



FINAL REPORT

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VALUE-ADDED BEEF PROGRAM

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College of Agriculture
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Value-Added Beef Program

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EXECUTIVE SUMMARY

In January of 2004, the Saskatchewan Beef Development Fund approved funding for an additional five years of support for the Value-added Beef Program (VAB) which was initiated in 1999. The objective of this program was to establish an applied research and new product development capability specifically for beef. Over the past six years, we have continued our value-added beef research and assistance to the beef industry. Most of the routine beef product development was provided by the Meat Group (Meat Service Project, formerly the Saskatchewan Food Product Innovation Program) with VAB personnel collaborating or assisting where appropriate.

There were several major projects completed over the past six years (Activity 1 to Activity 6). First, the effects of mechanical tenderization, moisture enhancement and enzyme treatment were evaluated on several cuts from the beef round. Then a large study was undertaken at the request of the Canadian Cattlemen's Association (CCA) on the effects of postmortem palatability enhancement strategies (moisture enhancement, blade tenderization, pre-tumbling) to improve the palatability of selected beef cuts from youthful and mature beef cattle (co-funded by the CCA). An industry type booklet was developed by the Beef Information Centre to feature this work and several seminars were organized by the CCA to relay the findings to the industry.

In addition to these Program thrusts, three graduate student projects received partial support from this program. A research project led by A. Perera, Ph.D. graduate student in the Department of Food and Bioproduct Sciences, examined the effects of thermal treatments to modify the toughness of beef connective tissue (co-funded by ADF). Of particular note was that connective tissue from mature cows had a much higher shear force (a measure of toughness) than that from heifers. This difference persisted even after extended aqueous heating. The program also contributed to a "proof of concept" study on the use of advanced drying technologies for beef jerky processing by V. Thiagarajan, a M.Sc. student in the Department of Agricultural and Bioresource Engineering. This study showed that newer drying technologies could be very efficient for drying, but further evaluations of the sensory properties are needed before recommendations can be made to the industry. More recently, L. Stoll, M.Sc. student in the Department of Food and Bioproduct Sciences received assistance for his project on the effects of distiller's grains on meat quality which was co-funded by the Agricultural Bioproducts Innovation Program through the Feed Opportunities for the Biofuel Industries (FOBI) network.

Overall, the core funding provided by the Beef Development Fund has allowed us to offer assistance to the beef industry and to maintain our expertise and infrastructure in the VAB area. The Value-Added Beef Program has been fortunate to find skilled personnel to meet its mandate. In particular, I would like to express my thanks to Dr. Zeb Pietrasik, who carried out or contributed to most of research projects described during his six-year tenure with the program, and who has continued to work with me on the related manuscripts. Further, the training of graduate students and program staff has contributed to the development of highly qualified personnel to meet Saskatchewan's future needs. We are very grateful to the Saskatchewan Beef Development Fund for its support over the past eleven years. This targeted source of funding for the beef industry will be missed.

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ACTIVITY 1 – POSTMORTEM INTERVENTION STRATEGIES TO ENHANCE TENDERNESS OF UNDERUTILIZED BEEF CUTS

1.1 Bovine *biceps femoris* is resistant to tenderization by lower-salt moisture enhancement or enzyme addition

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Abstract

Unacceptable toughness in retail beef cuts prepared from round muscles is common. The *biceps femoris* (BF) muscle is a particular challenge due to its high connective tissue content. Disruption of connective tissue by proteolytic enzyme injection has been demonstrated to improve tenderness in some muscles. Moisture enhancement can also be effective; however, concern over sodium content in processed foods is rising. The single and combined effects of lower-salt moisture enhancement (ME; 0.25% sodium chloride/0.25% sodium phosphate) and injection of enzymes from different sources (fungal aspartyl protease, bacterial protease, porcine pancreatin, plant-derived papain) on tenderness characteristics of the BF was examined. The enzyme and ME treatments were not interactive. ME had no impact on peak shear force or sensory tenderness, although juiciness and saltiness perception was enhanced, even at the relatively low salt level. Each enzyme treatment reduced the shear force associated with the myofibrillar component of the BF, but did not influence the connective tissue component. No off flavour development or other sensory defects resulted from enzyme treatment. The ineffectiveness of the treatments may have been due to the low salt level, lack of enzyme specificity for collagen, or the relatively intractable nature of the BF to tenderization treatments.

Keywords: Beef; tenderization; lower salt; moisture enhancement; enzyme injection; tenderness.

Abbreviations: BF, *biceps femoris* muscle. C, untreated control. AO, aspartic protease from *Aspergillus oryzae*. BS, neutral bacterial protease from *Bacillus subtilis*. PP, porcine pancreatin. PA,

purified papain. ME, moisture enhancement. NO, no application of moisture enhancement. WBSF, Warner-Bratzler shear force. SF-Myo, shear force related to myofibrillar protein. SF-CT, shear force related to connective tissue.

1.1.1. Introduction

Much research has been reported on the high level of unacceptable toughness in muscles and retail beef cuts originating from the round, especially when these cuts are cooked as steaks (Morgan et al. 1991; Brooks et al. 2000; Rhee et al. 2004; Von Seggern et al. 2005). Several studies that characterized a range of beef muscles have reported relatively high Warner-Bratzler shear force values and inferior juiciness and tenderness ratings for the *biceps femoris* muscle (Brooks et al. 2000; Nelson et al. 2004; Rhee et al. 2004; Kolle et al. 2004).

One approach to increase tenderness in lower value beef cuts is to substantially and selectively, disrupt the intrinsic collagen and elastin structure with the use of exogenous proteolytic enzymes in order to significantly reduce the amount of detectable connective tissue without causing extensive degradation of muscle fibres. Although enzyme tenderization of meat is a technique that has been available for many years (Thomas and Partridge 1960), widespread use of the method has been limited due to the potential for unsatisfactory results including ineffectiveness against connective tissue (Cronlund and Woychik 1986; 1987), and over-effectiveness in the myofibrillar fraction resulting in an undesirable mushy texture (Ashie et al. 2002; Stefanek et al. 2002) and off-flavours (McKeith et al. 1994).

Another approach that has been demonstrated to improve the eating quality of less tender beef cuts, and which aids in meeting consumer demand for higher quality and more convenient meat products is injection of a combination of ingredients such as sodium tripolyphosphate and sodium chloride (McGee et al. 2003). With moisture enhanced products becoming more readily available and accepted by consumers in the Canadian retail marketplace, it may now be practical to consider the addition of tenderizing agents to standard salt/phosphate marinades in order to further improve the eating quality of lower value beef cuts.

The objective of this study was to compare the effect of proteolytic enzymes of four different origins (plant, animal, microbial, and fungal) alone, and in combination with lower-salt moisture enhancement, on indicators of eating quality of bovine *biceps femoris*, a relatively low value muscle with high connective tissue content.

1.1.2 Methodology

1.1.2.1 Sample preparation and treatment application

Ten pairs of beef *biceps femoris* muscles (BF) were collected from young Canada Grade A or higher carcasses from the abattoir at the Agriculture and Agri-Food Canada Lacombe Research Centre. The carcasses were chilled to 0-2°C and then muscles were removed at 48 h postmortem. Muscles were vacuum packaged, aged at 3°C for 7 d, boxed and frozen in a blast-freeze. Until used, muscles were stored at -30°C.

All processing was carried out at <7°C. Vacuum packaged samples were thawed at 4°C for 72 hours. The flat side (posterior portion) of each muscle had a grain that was inconsistent with the fibre direction of the rest of the muscle, so it was removed along with the fat seam. The BF muscles were then trimmed of all visible fat and connective tissue, and five 600 ± 50 g roasts were cut from each muscle such that the grain of the roasts ran perpendicular to the cut surface.

Within each pair, the roasts from one muscle were assigned to one of five treatments: untreated beef (C), or injection with a water solution containing either aspartyl protease from *Aspergillus oryzae* (AO), ENZECO® neutral bacterial protease derived from *Bacillus subtilis* (BS), liquid porcine pancreatin (PP), or ENZECO® purified papain (PA). The selection of enzymes was based on the results of preliminary work. Roasts were injected with enzyme solutions to 105% of initial weight to achieve the following enzyme concentrations in the injected samples: 15 ppm (0.0015%) AO, 15 ppm (0.0015%) BS, 300 ppm (0.03%) PP, and 1 ppm (0.0001%) PA. Roasts from the second muscle in each pair received one of the following injection treatments: salt/phosphate moisture enhancement (ME), or a combination of moisture enhancement with an enzyme addition (ME+AO, ME+BS, ME+PP, ME+PA). These roasts were injected to achieve 12% extension (or 112% of initial weight), by weight, and to contain 0.25% sodium chloride and 0.25% sodium tripolyphosphate levels in the injected product. All meat injection was completed using a multi-needle injector (Model FGM 20/40, Fomaco Reiser Ltd., Burlington, ON, Canada). The roasts were weighed before and after injection to determine the percent brine pick up (injected weight-raw weight/raw weight x 100). Those roasts that did not reach their target weight were further injected with the same marinade using a hand injector to compensate for any deviation from the target weight.

All treatments were applied to each muscle pair, with location within the muscles balanced to ensure that all treatments were assigned twice to each location. All samples were vacuum packaged and

stored at 4°C. After about 48 h post-injection, samples were removed from packaging and two 2.54 cm thick steaks were prepared from each roast by cutting perpendicular to the muscle fiber orientation. The steaks were vacuum packaged, frozen in a blast-freezer, and stored at -30°C until evaluation.

1.1.2.2 Warner-Bratzler Shear Force (WBSF)

One steak from each roast was thawed at 4°C for 24 h and then cooked on an electric grill (Garland ED-30B electric grill; Condon Barr Food Equipment Ltd, Edmonton, AB, Canada). Steaks were placed on a grill preheated to 210°C, cooked until internal temperature reached to 35°C, turned over, and cooked until final internal temperature reached to 71°C. Cooking time and cooking loss [(raw weight at cooking - cooked weight / raw weight at cooking) x 100] were recorded for each steak.

Cooked steaks were allowed to cool at room temperature for approximately 2 h. The shear force of 7-9 core samples (1.27 x 1.27 x 2.54cm), cut parallel to the fibre direction from each steak, was determined. Samples were sheared perpendicular to the fibre direction using a TMS-2000 texture system (Food Technology Corp., Rockville, MD, USA) fitted with a Warner-Bratzler shear attachment. The maximum shear force (N) recorded during the test was reported as Warner-Bratzler shear force, while force-deformation curves from the Warner-Bratzler shear device were used to determine WBSF of the myofibrillar (**SF-Myo**) and connective tissue components (**SF-CT**). Namely, SF-Myo force was measured on the shear deformation curve as a peak value occurring within the first half of the distance the shear blade cut through the meat sample; whereas SF-CT was measured as a peak value within the last 3 mm before the shear blade had cut through meat sample. Total work done during sample shearing was calculated as force x distance the shear blade traveled to cut through the sample.

1.1.2.3 Sensory Properties

A sensory evaluation panel, consisting 8 members, trained and established as per published standards and guidelines of AMSA (1995) was employed for sensory analysis. Members of the panel were familiarized with the range of beef samples included in this study. They evaluated 5 samples per session over 20 evaluation sessions.

In preparation for each evaluation session, one steak from each treatment was thawed at 4°C for 24 h, and cooked as previously described for WBSF determinations. Cooked steaks were cooled at room temperature for 10 minutes and then eight 1.3 cm cubes were prepared from each steak, avoiding obvious connective tissue and large areas of fat. Cubes were randomly assigned to each panelist.

Samples were placed in glass jars in a circulating water bath and allowed to equilibrate to 68°C for 7 minutes prior to serving. The samples were presented to panelist according to a balanced design assigned by Compusense Five Software (v 4.6, Compusense, Inc., Guelph, ON, Canada) and attribute ratings were collected electronically on a 8-point descriptive scale for initial and overall tenderness [(8=extremely tender; 1=extremely tough), juiciness (8=extremely juicy; 1=extremely dry), beef flavour intensity (8=extremely intense; 1=extremely bland), salt intensity (8=extremely bland; 1=extremely intense), off flavour intensity (8=extremely bland, 1=extremely intense), and amount of connective tissue (8=none detected; 1=abundant)].

1.1.3 Results

No significant interactions were observed between moisture enhancement and enzyme treatments; therefore, only main effects are discussed. Neither moisture enhancement nor enzyme treatment had any influence ($p > 0.05$) on the cooking loss or time to reach the desired endpoint temperature (Figure 1.1.1).

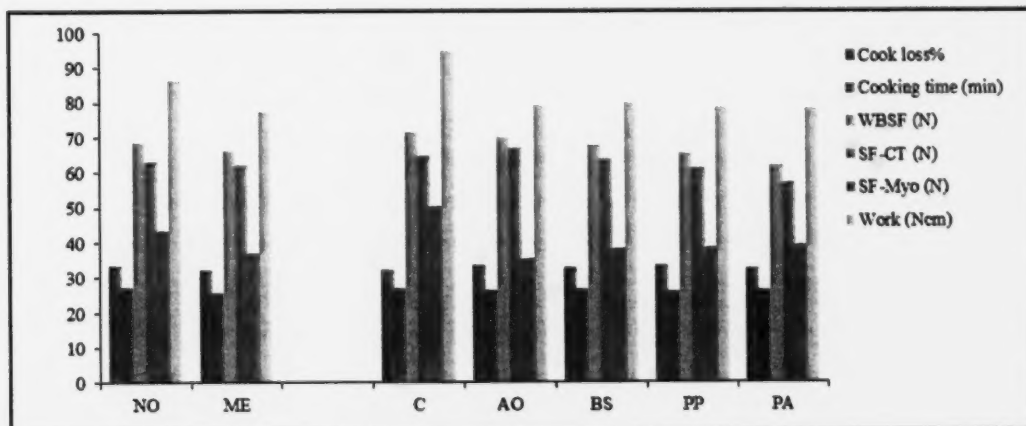


Figure 1.1.1 Effects of moisture enhancement and enzyme treatment on shear force parameters of *biceps femoris* steaks.

Abbreviations used are as follows: WBSF – peak shear force value; SF-CT – Connective tissue component of WBSF; SF-Myo – myofibrillar component of WBSF; N – Newton (SI unit of force, a newton can be converted into kg force by dividing the N value by 9.80665); Moisture enhancement treatments used are: steaks not enhanced with salt/phosphate solution (NO) and steaks injected with

salt/phosphate solution (ME). Enzyme treatments used are: control (C), 0.0015% of protease from *Aspergillus oryzae* (AO), 0.0015% of protease from *Bacillus subtilis* (BS), 0.03% of porcine pancreatin (PP) and 0.0001% of purified papain (PA).

1.1.3.1 Enhancement effect

Injection of BF muscles with the salt/phosphate brine did not affect ($p>0.05$) peak WBSF or SF-CT values (Figure 1.1.1). ME samples had lower SF-Myo shear values and also required less ($p<0.05$) energy to shear as compared to NO. Baublits et al. (2006b) demonstrated a linear effect of increasing salt concentration on decreasing shear values, beginning with at least 0.5% sodium chloride. This may mean that there could be a lower limit for salt content to achieve an adequate degree of tenderization with enhancement.

On the other hand, the lack of tenderization seen for the *biceps femoris* in the present study may have more to do with muscle type and composition. Previous researchers suggested that relatively large amount of collagen in the BF could be contributing to the lack of effect of moisture enhancement, indicating that the full tenderization effect of moisture enhancement may be muscle or muscle component specific.

1.1.3.2 Sensory evaluation

ME steaks were juicier than their non-enhanced counterparts (Figure 1.1.2), an effect consistent with other published reports on the influence of moisture enhancement on palatability attributes of beef (Vote et al. 2000; McGee et al. 2003; Robbins et al. 2002). Despite the lower salt concentration targeted, the ME treatment was sufficient to significantly increase saltiness. Unexpectedly, no differences were found between NO and ME for the other sensory attributes (Figure 1.1.2); however, the lack of difference in tenderness or perceived connective tissue amount is supported by shear force data.

Table 1.1.1 shows the simple correlation coefficients between instrumental and sensory tenderness parameters. All sensory tenderness parameters were correlated to SF-CT but had no significant relationship with SF-Myo. This seems to indicate that the connective tissue component was the driving factor related to sensory tenderness of the BF muscles. Since ME had no effect on SF-CT, it was not surprising that there was no detectable treatment effect of ME on sensory tenderness scores.

Obuz et al. (2003) reported SF-CT force was the main component for biceps femoris and suggested that the higher correlation coefficient between SF-CT and WBSF likely was due to the more pronounced effect of connective tissue in *biceps femoris* muscle.

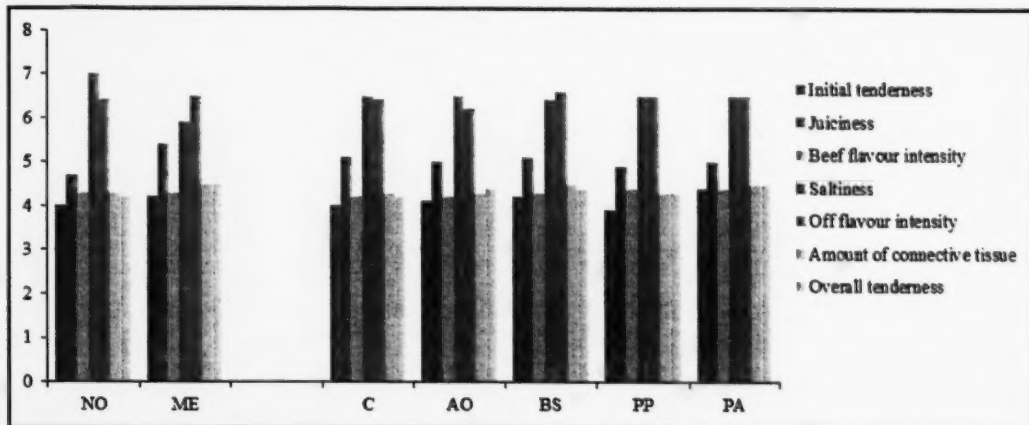


Figure 1.1.2 Effect of moisture enhancement and enzyme treatments on sensory characteristics of *biceps femoris* steaks.

Abbreviations used are as follows: WBSF – peak shear force value; SF-CT – Connective tissue component of WBSF; SF-Myo – myofibrillar component of WBSF; N – Newton (SI unit of force, a newton can be converted into kg force by dividing the N value by 9.80665); Moisture enhancement treatments used are: steaks not enhanced with salt/phosphate solution (NO) and steaks injected with salt/phosphate solution (ME). Enzyme treatments used are: control (C), 0.0015% of protease from *Aspergillus oryzae* (AO), 0.0015% of protease from *Bacillus subtilis* (BS), 0.03% of porcine pancreatin (PP) and 0.0001% of purified papain (PA).

In our study correlation coefficients between WBSF and SF-CT (Table 1.1.1) were similar that those reported in Obuz et al. (2003) study for *biceps femoris* muscle; however, the relationship we observed between WBSF and SF-Myo seemed to be dependant on moisture enhancement. The shear force associated with the connective tissue component was found to correlate to the maximum WBSF of both NO and ME samples, whereas SF-Myo was correlated to the peak WBSF only in non-enhanced steaks. This indicates that moisture enhancement with salt/phosphate brine mainly targets changes in myofibrillar proteins without affecting connective tissue, even when an overall treatment effect is

absent. So, it appears that the beneficial effect of moisture enhancement results from reduction of the myofibrillar component toughness, hence injection with salt/phosphate solution may be ineffective for improvement of palatability characteristics in muscles with high connective tissue content.

Table 1.1.1 Simple correlation coefficients between sensory tenderness and instrumental shear force parameters for non-enhanced control (NO) and moisture enhanced (ME) biceps femoris steaks

Non enhanced (n=50)						
	SF-CT	SF-Myo	WBSF	Initial tenderness	Connective tissue amount	Overall tenderness
SF-CT		0.19	0.96**	-0.55**	-0.57**	-0.58**
SF-Myo	0.19		0.36*	-0.21	-0.14	-0.19
WBSF	0.96**	0.36*		-0.58**	-0.57**	-0.59**
Initial tenderness	-0.55**	-0.21	-0.58**		0.89**	0.92**
Amount of connective tissue	-0.57**	-0.14	-0.57**	0.89**		0.94**
Overall tenderness	-0.58**	-0.19	-0.59**	0.92**	0.94**	
ME (n=50)						
	SF-CT	SF-Myo	WBSF	Initial tenderness	Connective tissue amount	Overall tenderness
SF-CT		0.14	0.99**	-0.57**	-0.53**	-0.55**
SF-Myo	0.14		0.21	-0.00	-0.03	-0.02
WBSF	0.99**	0.21		-0.53**	-0.49**	-0.51**
Initial tenderness	-0.57**	-0.00	-0.53**		0.92**	0.94**
Amount of connective tissue	-0.53**	-0.03	-0.49**	0.92**		0.94**
Overall tenderness	-0.55**	-0.02	-0.51**	0.94**	0.94**	

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

1.1.3.3 Enzyme tenderization effect

Enzyme injection did not affect peak shear force or the connective tissue component of WBSF; however, each of the four enzyme treatments significantly ($p < 0.05$) reduced myofibrillar component of WBSF and total energy required to shear (Figure 1.1.1). This suggests that none of the enzymes was active against the targeted insoluble connective tissue and degraded only the myofibrillar fraction of the BF muscles. No differences were found between control and enzyme-treated *biceps femoris* samples for any sensory attribute (Figure 1.1.2). Although this indicates that the samples were not tenderized in any meaningful way, it is important to note that there was also no development of off flavors or other defects sometimes associated with enzyme addition. Unlike some of the broad-spectrum enzymes derived from plant sources, it is believed that the aspartic protease expressed in

Aspergillus oryzae has very limited specificity on meat proteins and acts only on myofibrillar proteins, but not connective tissue (Ashie et al. 2002). Although papain has been reported to digest insoluble meat collagen (Cronlund and Woychik 1986), its predominant action is proteolysis of myofibrillar proteins, especially actin and myosin. Kang and Rice (1970) reported that papain solubilized 15% of connective tissue protein as compared to a 60% degradation of salt-soluble protein.

1.1.3.4 Location effect

The *biceps femoris* muscles included in this study showed significant ($p < 0.05$) variation in shear force parameters (Figure 1.1.3) and sensory tenderness (data not shown) across the sampling locations. WBSF and SF-CT values peaked in a middle region of the muscle with values decreasing towards the anterior and posterior portions of the muscle. This locational effect was not reflected in values obtained for the myofibrillar component of shear force (SF-Myo), which were similar across sampling locations.

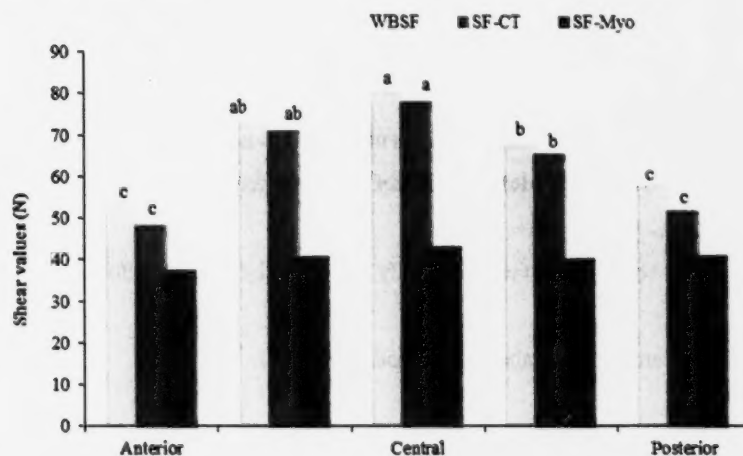


Figure 1.1.3 Effect of location within biceps femoris muscle on shear force parameters. Columns without a common letter are significantly different ($P < 0.05$).

1.1.4 Conclusions

Although enzyme injection did not cause extensive degradation of muscle fibres or adverse effects on sensory attributes related to palatability, none of the enzymes tested was able to significantly improve sensory tenderness or reduce the amount of detectable connective tissue in BF muscle. All four enzymes significantly reduced the shear force associated with the myofibrillar component of the

muscle, but none had any effect on peak shear force or the force associated with the connective tissue component of shear. Moisture enhancement with brines containing salt/phosphate improved juiciness and diminished the contribution of myofibrillar toughening to overall shear force. Salt/phosphate injection appears to have no effect on other palatability of BF, particularly when lower salt concentrations are used. Our results also indicate that location within the BF muscle can affect the results of tenderness evaluations and should be considered when designing experiments and interpreting results.

Acknowledgements

Financial support for this study by the Canadian Beef Cattle Research Council and by the Saskatchewan Beef Development Fund through the Value-added Beef Program is gratefully acknowledged. Most meat for these studies and some of the sensory evaluation costs were graciously donated by the Agriculture and Agri-Food Canada Lacombe Research Centre. Thanks are also expressed to Lorna Gibson and the sensory panel at the Lacombe Research Centre for their efforts, and to Neil Wiebe from the Meat Group at the University of Saskatchewan for technical assistance.

1.2 Influence of blade tenderization, moisture enhancement and pancreatin enzyme treatment on the processing characteristics and tenderness of beef *semitendinosus* muscle

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Abstract

The combined effect of blade tenderization (BT), moisture enhancement and enzymatic tenderization on drip loss, cook loss, Warner-Bratzler shear force (WBSF) and sensory characteristic of beef *semitendinosus* (ST) steaks from young cattle under 30 months of age was investigated.

Injection with phosphate/sodium chloride solution improved tenderness and juiciness of *ST* muscles ($P<0.01$). No additional improvement in tenderness was observed with incorporation of a pancreatin enzyme preparation into the moisture enhancement solution ($P>0.1$). Injection of pancreatin alone tended to improve overall tenderness ($P=0.09$) and did not adversely affect other palatability attributes. Blade tenderization of *ST* muscles improved tenderness, as indicated by lower WBSF and increased sensory tenderness scores than control samples, without decreasing flavor and juiciness. Our results suggest that moisture enhancement and blade tenderization can be effectively utilized to reduce the variability in and improve both tenderness and palatability of *ST* muscles. Pancreatin was not particularly effective at the 0.02% level used.

Keywords: Beef; Tenderization; Moisture enhancement; Enzyme injection; Tenderness.

1.2.1. Introduction

Tenderness is an important driver of beef consumer satisfaction and as such has been extensively investigated (Boleman et al., 1997; Neely et al., 1998; Beef Information Centre, 2002). Many research reports indicated a high level of unacceptably tough retail cuts originating from the round, especially when cooked as steaks (Morgan et al., 1991; Brooks et al., 2000; Rhee, Wheeler, Shackelford & Koohmaraie, 2004; Von Seggern, Calkins, Johnson, Brickler & Gwartney, 2005).

While relatively simple intervention strategies can be effective for tenderness enhancement in muscles such as the *longissimus*, the *semitendinosus* (*ST*) is resistant to tenderization due to a high elastin content (Bendall 1967; Nguyen & Zarkadas 1989) and as a consequence is retailed as a relatively low value steak (Beef Information Centre, 2002; Brooks et al. 2000). Moderate improvement of tenderness in *ST* muscles has been previously reported with application of blade tenderization (Seideman, Smith, Carpenter & Marshall (1977). Proteolytic enzymes, particularly with sufficient specificity to target elastin, may be a possible option for *ST* enhancement. Enzymatic tenderization of meat has been used for many years using enzymes of plant origin such as papain, bromelin, and ficin. However, many tenderizing enzymes lack substrate specificity and without careful control over application and action, overtenderization may result causing an undesirable mushy, pasty texture (Ashie, Sorensen & Nielsen, 2002; Stefanek, Scanga, Belk & Smith, 2002) and off-flavours (McKeith, Brewer, & Bruggen, 1994).

The objective of the current study was to investigate the effect of salt/phosphate moisture enhancement and pancreatin enzyme treatment in combination with blade tenderization (BT) on the cooking properties and palatability of beef ST muscles.

1.2. 2 Methodology

Sixteen paired beef semitendinosus muscles (ST) from Canada Grade A carcasses were obtained from the Agriculture and Agri-Food Canada Lacombe Research Centre (Lacombe, AB, Canada) abattoir. All muscles were removed from carcasses at 48 h postmortem following conventional carcass chilling (0-2°C), then vacuum packaged, aged to 7 d postmortem at 3°C, boxed and frozen in a blast-freezer. Until process muscles were stored at -30°C. An elastase-containing food-grade enzyme cocktail designed for cheese making (liquid porcine pancreatin; RENCO New Zealand, Eltham, NZ; distributed by Danlac, Airdrie, AB) was chosen for injection.

1.2.2.1. Preparation of beef steaks

All processing was carried out at <7°C. Before use muscles were thawed at 3°C for 72 h. Then muscles were trimmed of visible, external fat and connective tissue, and cut transversely into two 1.0 kg sections, designated distal and proximal. Three water-based injection solutions were prepared: an enzyme treatment (ENZ), a moisture enhancement treatment (ME), and a combined moisture enhancement with enzyme treatment (ME+ENZ). The enzyme treatment was formulated to give 0.02% porcine pancreatin in the final injected product. Brines for moisture enhancement were formulated to give 0.5% sodium chloride and 0.25% sodium in injected meat. The combined ME +ENZ treatment were formulated to give 0.02% porcine pancreatin, 0.5% sodium chloride and 0.25% tripolyphosphate.

The roasts designated for ENZ treatment were injected to a target weight of 5% (is it 5% or should be 105%?) of the original weight, while the roasts designated for ME and ME+ENZ treatments were injected to achieve 15% extension. All treatments were allocated to muscle locations such that each location/injection treatment combination was present and replicated. Meat injection was completed using a Reiser Fomaco multi-needle injector (Model FGM 20/40, Fomaco Reiser Ltd., Burlington, ON). The roasts were weighed before and after injection to determine the percent brine pick up (i.e., injected weight-raw weight/raw weight x 100). Those muscles that did not reach their target weight were further injected with the same marinade using a hand injector to compensate for any deviation from the target weight.

Injected muscles and non-injected controls (C) were then divided transversely through the center into approximately equal halves. Then half of the roasts from all controls and treatment groups were blade tenderized by one pass through a Jaccard®, Model H # B4590 meat tenderizer (Jaccard®, Orchard Park, NY, USA). All muscle samples were vacuum packaged and stored at 4°C. After 48 h, samples were removed from packaging and weighed to determine the retained injection level $[(\text{weight at 48 h/raw weight}) \times 100]$ and drip loss $\{[(\text{injected weight before 48 h storage} - \text{weight at 48 h/injected weight before 48 h storage}) \times 100]$. Two 2.54 cm thick steaks were obtained by cutting perpendicular to the muscle fiber orientation. The steaks were vacuum packaged, frozen in a blast-freezer and stored at -30°C until evaluation.

1.2.2.2 Warner-Bratzler shear force (WBSF)

Steaks were thawed to 4°C and placed on an electric grill (Garland ED-30B electric grill; Condon Barr Food Equipment Ltd, Edmonton, AB), preheated to 210°C. Steaks were cooked to reach the internal temperature to 35°C, turned over and cooked until final internal temperature reached 71°C. Cooking yield $[(\text{cooked weight} / \text{weight before cooking}) \times 100]$, was recorded for each steak. Cooking time for each steak to reach 71°C internal temperature was also calculated and expressed as min/100 g of sample.

After cooking, steaks were cooled to room temperature. Shear force of 7-9 core samples (1.27 x 1.27 x 2.54cm), cut parallel to the fibre direction, was determined. Samples were sheared perpendicular to the fibre direction using a TMS-90 texture system (Food Technology Corp., Rockville, MD, USA) fitted with a Warner-Bratzler shear attachment. Force-deformation curves were used to determine WBSF myofibrillar component (SF-M) and WBSF connective tissue component (SF-C). The height of the first peak corresponded to SF-M and the height of the second peak corresponded to SF-C. Maximum peak force recorded during the test was reported as Warner-Bratzler shear force.

1.2.2.3 Sensory properties

Steaks were thawed to 4°C and cooked as previously described for Warner-Bratzler shear force determination. After cooling for 10 minutes, cooking losses were determined by weight difference. Each steak was cut into 1.3 cm cubes, avoiding areas of connective tissue and fat. Eight cubes from each sample were randomly assigned to an eight-member trained taste panel. Samples were pre-equilibrated to 68°C in a circulating water bath prior to serving. The samples for each panelist were presented in a balanced design assigned by Compusense 5 Software, version 4.6 (Compusense Inc., Guelph, ON). Attribute ratings were electronically collected using the Compusense 5, release 4.6

software using an eight-point descriptive scales for initial and overall tenderness (8 = extremely tender; 1 = extremely tough), juiciness (8 = extremely juicy; 1 = extremely dry), beef flavour intensity (8 = extremely intense beef flavour, 1 = extremely bland beef flavour), salt intensity (8 = extremely bland; 1 = extremely intense), off flavour intensity (8 = extremely bland/no off flavour; 1 = extremely intense off flavour), and amount of connective tissue (1 = abundant; 8 = none detected). The panelists were instructed to describe off-flavors as bitter, bloody/serum, fatty, livery, metallic, salty, soapy, or sour, or using their own descriptors.

1.2.3. Results

No significant interactions were observed between injection treatments and blade tenderization; therefore, only main effect means are presented.

1.2.3.1. Brine retention and purge

Drip losses from the muscles 48 h post-enhancement indicated that ME and ME+ENZ treatment groups were close to the 115% target level while retained injection level in the ENZ samples were well below the target (Figure 1.2.1).

Following injection to the target of 105%, the weight of ENZ samples declined to 97.6% of raw weight, demonstrating a loss of a portion of the original sample weight in addition to that gained immediately following injection. Drip loss during vacuum-packaged storage represents the degree to which the meat was able to retain the added moisture from the injection. It was higher for the ENZ treatment than ME and ME+ENZ treatments although the loss following enzyme injection was not significantly different from C (Figure 1.2.1). In the present study and that of Seideman et al. (1977) drip loss from ST muscle was not affected by a single pass through a blade tenderizer.

1.2.3.2. Cooking properties

Moisture enhanced steaks with or without enzyme treatments needed significantly ($P < 0.05$) less time (~9 min/100 g) to reach the endpoint temperature (71°C) compared to the non-injected counterpart (~11 min/100 g). The cooking loss of ST steaks from ME and ME+ENZ treated samples was significantly ($p < 0.001$) higher than that of C and ENZ samples (Figure 1.2.1). This was expected, as higher amounts of water retained due to the salt/phosphate in the brine for ME (15%) samples compared to ENZ treatment (5%). Blade tenderization significantly ($P < 0.05$) increased the cooking losses (Figure 1.2.1). Although present data were obtained from the injected muscles our results support some of the previous findings (Davis, Smith & Carpenter, 1977).

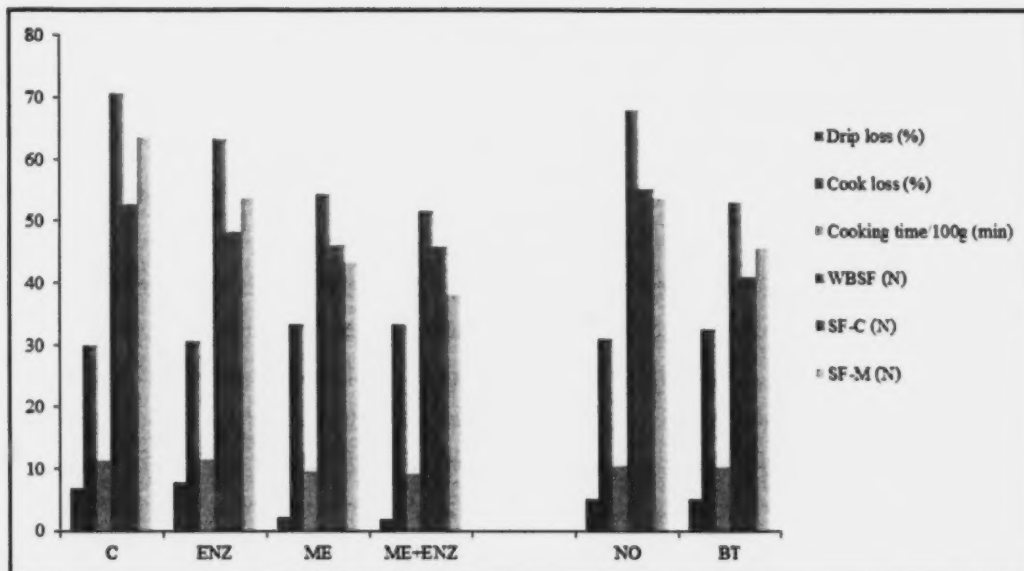


Figure 1.2.1 Effect of injection treatments and blade tenderization on processing and textural properties of ST steaks.

Abbreviations used are as follows: WBSF – peak shear force value; SF-C – connective tissue component of WBSF; SF-M – myofibrillar component of WBSF (N = newton, SI unit of force and a newton can be converted into kg force by dividing the N value by 9.80665). Injection treatments used are: non-injected – Control or C, enzyme injection – ENZ, moisture enhanced injection – ME and moisture enhanced with enzyme injection – ME + ENZ. Blade tenderization treatments used are: not tenderized – NO and blade tenderized – BT.

1.2.3.3 Warner-Bratzler shear force

Injection of brine containing salt/phosphate (ME and ME+ENZ) into ST muscles significantly ($P < 0.001$) reduced WBSF and SF-M values compared to C samples. There was a tendency ($P = 0.08$) for the ENZ treatment (0.02%) to have lower WBSF than C control samples (Figure 1.2.1). Enzyme treatments (ME+ENZ and ENZ) had lower SF-M values ($P < 0.05$) compared to C samples. However, no additional reduction in WBSF and SF-M was observed after enzyme injection in moisture

enhanced muscles (ME versus ME+ENZ), which suggests that moisture enhancement solution alone may have reduced WBSF to acceptable levels. Application of blade tenderization substantially decreased all WBSF values (Figure 1.2.1). WBSF results were well supported by sensory evaluation scores.

1.2.3.4 Sensory evaluation

Results from the sensory panel for the main effects of injection treatments and blade tenderization are reported in Figure 1.2.2. Moisture enhancement, with or without enzyme addition, produced the largest increase in overall tenderness scores of grilled beef steaks compared to controls (6.4, 6.2 and 5.3, respectively; $P < 0.01$). No differences were found between C and ENZ treated samples for any palatability trait. The use of pancreatin did not adversely affect any palatability attributes but also was not able to significantly reduce the amount of connective tissue detected by the sensory panelists. In contrast, WBSF results showed enzyme injection significantly reduced the SF-M without affecting SF-C values. These results suggest that pancreatin injection was not exclusively significantly active against the targeted insoluble connective tissue in the meat matrix. However, lack of significant tenderization by pancreatin is most likely to be due to low levels of retention of the enzyme in meat, limited distribution of the enzyme beyond the point of injection in meat matrices or ? in this study. However, we might expect that if higher concentrations of pancreatin are used, some deterioration of palatability could be possible.

Both phosphate/sodium chloride treatments (ME and ME+ENZ) produced beneficial effects ($P < 0.05$) on tenderness and juiciness compared with controls and samples injected with enzyme solution alone. The beneficial effect of phosphate/sodium chloride injection on sensory tenderness and juiciness shown in our study was consistent with other published reports (Vote et al., 2000; Robbins et al., 2002; McGee et al., 2003). Understandably, addition of the phosphate/sodium chloride solutions increased panel ratings for perceived salty flavor compared to untreated controls and injection with enzyme only. Consequently, treatments that included salt were judged to have less ($P < 0.05$) beef flavor than the control or enzyme treatments. However, it must be kept in mind an experienced trained panel, which performed the evaluations in the present study, may be more sensitive to salt than the general population. Blade tenderization also improved ($P < 0.05$) the initial and overall tenderness and made the connective tissue less perceptible. However, it tended to reduce juiciness ($P = 0.09$), but did not adversely affect other palatability attributes. Sensory panel results and WBSF data suggest that both phosphate/ sodium chloride treatments (ME and ME+ENZ treatments) and BT improved tenderness of ST and these methods can be used together to further improve tenderness.

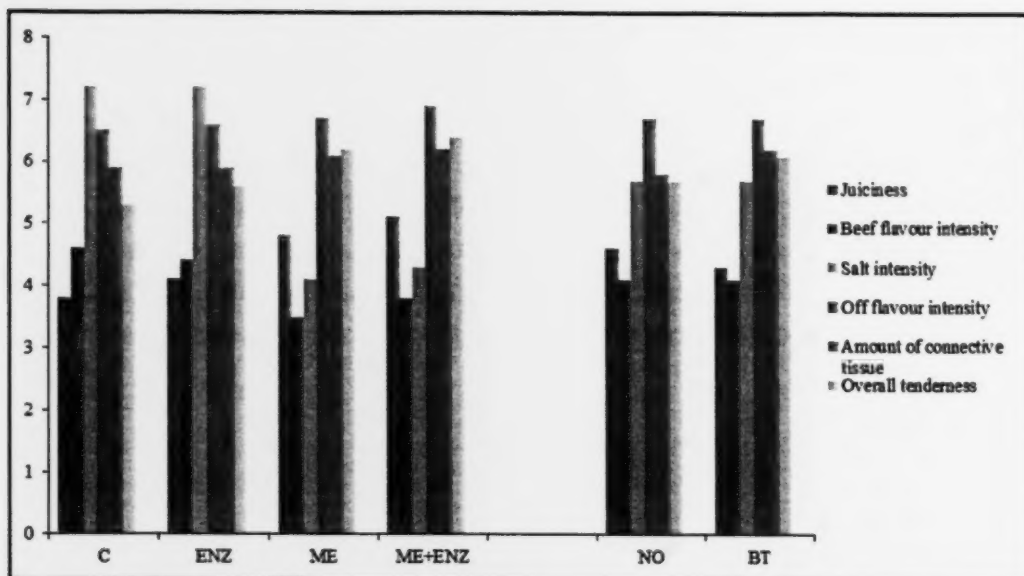


Figure 1.2.2 Effects of injection treatments and blade tenderization on sensory panel traits of ST steaks.

Sensory scores are based on 8 point scales (8 = extremely tender, juicy, intense, bland, none, tender and 1 = extremely tough, dry, bland, intense, intense, abundant). Injection treatments used are: not non-injected or control – C, enzyme injection – ENZ, moisture enhanced injection – ME and moisture enhanced with enzyme injection (ME + ENZ). Blade tenderization treatments used are: not tenderized – NO and blade tenderized – BT.

1.2.4. Conclusions

Results of this study indicated that injection of phosphate/sodium chloride solution to achieve 15% extension in beef ST is a potential way of improving tenderness and juiciness of ST muscle. Injection of porcine pancreatin tended to reduce WBSF ($P = 0.08$) and improve overall tenderness ($P = 0.09$) without decreasing flavor and juiciness. As pancreatin was not able to significantly reduce the amount of detectable connective tissues it suggests that pancreatin is not effective against the connective tissue. Blade tenderization can be effectively utilized to improve initial and overall tenderness and reduce the amount of perceptible connective tissue in the ST muscle. Also, combined

application of ME and ME+ENZ treatments would provide enhanced tenderness as compared to using these methods individually.

Acknowledgements

Financial support for this study by the Canadian Beef Cattle Research Council is gratefully acknowledged. Partial funding for this project was also provided by the Saskatchewan Beef Development Fund through the Value-added Beef Program. Most meat for these studies and some of the sensory evaluation costs were graciously donated by Agriculture and Agri-Food Canada, Lacombe Research Centre. Thanks are also expressed to the sensory panel at the Lacombe Research Centre for their efforts and Neil Wiebe from the Meat Group at the University of Saskatchewan for technical assistance.

1.3 Influence of blade tenderization, moisture enhancement and pancreatin enzyme treatment on the processing characteristics and tenderness of beef semitendinosus muscle

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Status:

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Abstract

The combined effect of blade tenderization (BT), moisture enhancement and enzymatic tenderization on drip loss, cook loss, Warner-Bratzler shear force (WBSF) and sensory characteristics of beef semitendinosus (ST) steaks from cattle under 30 months of age was investigated. Injection with phosphate/sodium chloride solution improved tenderness and juiciness of ST muscles ($P < 0.01$). No additional improvement in tenderness was observed with incorporation of a pancreatin enzyme preparation into the moisture enhancement solution ($P > 0.1$). Injection of pancreatin alone tended to improve overall tenderness ($P = 0.09$) and did not adversely affect other palatability attributes. Blade tenderization of ST muscles improved tenderness, as indicated by lower WBSF and increased sensory tenderness scores than for control samples, without decreasing flavor and juiciness. The results

suggest that moisture enhancement and blade tenderization can be effectively utilized to reduce the variability in and improve both tenderness and palatability of ST muscles. Pancreatin was not particularly effective at the 0.02% level used in reducing amount of detectable connective tissues.

Acknowledgements

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ACTIVITY 2 – EFFECT OF POSTMORTEM TENDERIZATION STRATEGIES (ENHANCEMENT, BLADE TENDERIZATION AND OTHER TENDERIZATION METHODS) ON PROPERTIES OF SELECTED BEEF MUSCLES FROM YOUTHFUL AND MATURE BEEF CARCASSES.

2.1 Researching tenderization methods for commercial beef

Status: This is a modified version of the industry information brochure, 'Commercial Beef New Markets' published by the Beef Information Centre (processing@beefinfo.org). A PDF file of the brochure is enclosed. A more detailed manuscript has also been drafted and will be submitted to the Journal of Animal Science in the near future.

2.1.1 Introduction

Canada's beef industry has been subject to various trade restrictions since the first case of BSE was confirmed in 2003. Although the United States recently lifted its ban on older cattle imports, it will take some time before the export of mature live cattle and their beef products returns to pre-BSE levels. The resulting expansion of available D-grade (commercial) beef from mature (over-thirty-month) cows in Canada therefore provides a strong incentive for the Canadian meat industry to find more profitable methods for utilizing commercial beef. Even as the border is reopened, such methods can continue to provide the Canadian beef industry with increased rates of return on these cattle. Canada has over 5.3 million beef cows, and normally culls between eight and ten per cent each year. Even modest improvements in the processing of mature cattle to create higher-valued beef products would result in millions of dollars of benefit to the beef industry.

Previous research has shown while the flavour and juiciness of commercial beef is often equivalent, or even superior, to beef from youthful cattle, its tenderness is inferior. As a result, it has failed to provide customer satisfaction. If, however, postharvest tenderization techniques are effective at improving the acceptability of mature cuts, it may be possible to use these techniques to increase the overall marketability of commercial beef. By enhancing the utilization and value of beef from older culled cattle, this may also enhance profitability of the whole Canadian beef industry.

2.1.2 Methodology

2.1.2.1 Sample Preparation

All carcasses were graded by a certified grader. Overall, there were twelve Canada D1 cows, twelve Canada D2 cows, and twelve Y1 beef heifers graded either Canada AA or AAA. After slaughter, all carcasses were chilled for 48 hours at 4°C. Then pairs of all four muscle cut categories (strip loin, bottom round, top round, top butt) were collected from each animal (a total of 288 muscles), vacuum-packaged, and aged for 12 days at 3°C. After aging, each muscle was completely denuded (freed from external fat and connective tissue). Since properties of a muscle may change from one end to the other, the researchers divided each muscle in three sections by cutting across the grain (or along the fat seam in the case of top portion of top butt cuts). Cuts were then subjected to the tenderization treatments listed below.

No treatment – C

Blade tenderization – BT (Passed once through a blade tenderizer)

Pre-tumbling – PT (Pre-tumbled for 30 minutes, no vacuum)

Moisture enhancement – ME (Multi-needle injected to 112% raw meat weight with defined brine containing sodium chloride and sodium tripolyphosphate)

Blade tenderization, then moisture enhancement – BT + ME (BT and ME combined)

Pre-tumbling, then moisture enhancement – PT + ME (PT and ME combined)

Due to the inherent differences in meat properties from different animals, the experiment was designed so that all six treatments could be applied to each of the meat cuts from an individual animal. After treatment, all muscle sections were vacuum-packaged and stored for an additional 48 hours at 4°C. At this point, the roasts were unpacked, weighed to determine purge, cut into 2.5 cm (1 inch) steaks, frozen in a blast-freezer and stored at -30°C until evaluation. Steaks were thawed at 4°C and cooked on an electric grill. To ensure uniformity, the internal temperature of each steak was monitored closely. Steaks were turned when the internal temperature reached 35°C and removed from the heat when the final internal temperature reached 71°C.

2.1.2.2 Attributes Tested – Instrumental

Proximate analysis: An analysis of total moisture, crude protein, and total collagen was performed. Muscles containing more collagen are generally less tender.

pH analysis: Meat pH, which is a measure of acidity, is a good indication of water holding capacity.

Purge during storage: Purge was determined by weighing roasts prior to the 48-hour storage period and just before cutting and freezing.

Warner-Bratzler Shear Force (WBSF): The force required to shear through a standard core of meat (1.27 cm x 1.27 cm) at right angles to the grain. WBSF is a good measurement of tenderness; it was performed on cooked steaks (see WBSF device on previous page).

Expressible moisture: This is determined by expressing liquid from a small steak sample using a specialized device. Steaks with more expressible moisture tend to receive higher juiciness scores in consumer tests, although many factors play a role when it comes to juiciness.

2.1.2.3 Sensory attributes

Trained panel evaluations: A trained and experienced eight-member sensory panel evaluated initial and overall tenderness, juiciness, flavour intensity, connective tissue amount and off-flavour intensity. The panel used an 8-point scale to characterize the samples. In addition, the panel was asked to describe any off-flavours present (e.g., bitter, metallic, salty, etc.).

Consumer panel testing: In the follow-up phase II trial, consumer panelists evaluated steak flavour, tenderness, juiciness and overall acceptability using 9-point scales. Panelists were also asked to indicate purchase intent. Sensory analysis of samples was performed at Agriculture and Agri-Food Canada in Lacombe, Alberta and the Consumer Product Testing Centre, Alberta Agriculture and Food in Edmonton, Alberta.

2.1.3 Results

2.1.3.1 Instrumental testing

Strip Loin (*Longissimus lumborum*): All treatments were able to reduce Warner-Bratzler Shear Force (WBSF) in strip loin steaks. BT + ME and PT + ME were most effective, with all treatments capable of yielding steaks from mature muscles of comparable tenderness to those of untreated youthful muscles.

Bottom Round (*Biceps femoris*): BT and especially BT + ME were able to appreciably reduce WBSF in bottom round steaks. As a matter of fact, BT + ME was capable of yielding steaks comparable in tenderness to those of untreated youthful muscles.

Top Round (*Semimembranosus*): All interventions proved successful in improving tenderness of top round steaks. However, BT + ME was most effective. Some treatments, notably ME-only, PT + ME and BT + ME were able to improve tenderness to a level comparable to that of untreated youthful muscles.

Top Butt (*Gluteus medius*): All interventions were capable of reducing WBSF in top butt steaks. BT + ME and PT + ME resulted in the most tender steaks. However, in general, all of the interventions were capable of improving tenderness of steaks from mature animals to the same level as from untreated youthful muscles.

2.1.3.2 Sensory testing

Similar to the findings obtained through instrumental measurements, BT + ME was able to enhance the tenderness of the four mature muscles tested to the level of untreated (C) youthful muscles. However, the overall tenderness of steaks from mature muscles ranged from 4.0 in the bottom round to 5.7 - 5.8 in the strip loin and top butt (8 = extremely tender; 1 = extremely tough). Thus, post-harvest tenderization strategies are muscle specific. There was good success for strip loins and top butt from mature animals. While scores for bottom round were improved, the bottom round from mature animals may not have achieved sufficient tenderness for consumer acceptability. (For data please refer to the attached PDF file 'Commercial Beef New Markets')

None of the treatments were capable of achieving sufficient tenderness in every steak; however, the number of steaks rated as slightly tender or higher increased significantly after BT + ME. Finally, the tenderness ratings did not differ between muscles derived from D1 and D2 cattle.

2.1.4 Summary

Several muscles from mature beef carcasses have been identified as failing to provide consumer satisfaction. The current study was undertaken to investigate the impact of processing strategies (blade tenderization [BT], pre-tumbling [PT], and moisture enhancement [ME]), alone and in combination, on processing yield and eating quality-related parameters of selected loin and hip

muscles (biceps femoris [BF], gluteus medius [GM], longissimus lumborum [LL], and semimembranosus [SM]) from youthful and mature beef cattle. Results indicate that muscles from mature beef were inherently less tender, but that processing treatments could improve quality, primarily on a muscle-specific basis. Of the different tenderization strategies evaluated, BT followed by ME by injection of a salt/phosphate solution was the most effective strategy for improving tenderness of all the muscles examined, although no additional tenderization of GM and LL samples was observed by combining PT or BT with ME. Combining PT or BT with ME, however, was effective to control the increase purge loss observed following ME treatment. Pre-tumbling as a single treatment was ineffective in all of the muscles, and only treatments that included BT were sufficient to effect an increase in tenderness of BF.

Acknowledgements

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ACTIVITY 3 - STRATEGIES TO MODIFY CONNECTIVE TISSUE AND ENHANCE TENDERNESS OF MATURE BEEF

Status: This article is a modified version of a part of the PhD Thesis published by Anula Perera.
(http://library2.usask.ca/theses/available/etd-03252009-4013/unrestricted/Anula_Perera_Thesis.pdf).

3.1 Dynamics of bovine epimysial properties subjected to aqueous heating

3.1.1 Introduction

Cooking has a major influence on the tenderness of a meat product as the water- and fat-binding characteristics, as well as the texture, are closely related to the conditions of heating. Selecting an appropriate heating rate is important to determine the quality of final product, and is particularly important for lesser value cuts with high levels of connective tissue.

Myofibrillar and connective tissue proteins (mainly collagen) are responsible for major textural changes in meat during heating (refer to activity 1). It is accepted that heat solubilises connective tissue collagen and that results in meat tenderization whereas heat denatures myofibrillar proteins and that results in meat toughening. These heat-induced changes are time and temperature dependent.

Myofibrillar proteins exposed to thermal energy (heating) are denatured and that results in protein coagulation and loss of solubility. Collagen of the connective tissue undergoes physical changes during heating, i.e. swells, in the presence of moisture, and shrinks. With extended heating in the presence of moisture, collagen is further hydrated and hydrolyzed, to make meat tender. During cooling, soluble collagen forms a gel network. As such, heat causes some tenderization and toughening of meat simultaneously due to its variable effect on different constituents of meat proteins.

Changes that take place in connective tissue embedded in the muscle matrix were previously studied. Studying the thermal behaviour of connective tissue per se was considered a useful exercise to understand the true nature of changes that take place in connective tissue in the muscle matrix. In order to obtain sufficient material of uniform thickness to work with, a decision was made to replace perimysium (as originally planned) with epimysium.

3.1.2 Methodology

3.1.2.1 Epimysium sample preparation and treatments

Locally purchased bovine *longissimus* muscles from 4 heifers (17 months) and 4 cows (60-88 months) were used in this experiment. The dorsal epimysium with a layer of meat was excised on the 7th day post-mortem and stored at -20°C. After removing fat and muscle fibres, two adjacent strips of epimysium were cut along the length to prepare 2 x 2½ cm pieces (1.4 ± 0.4 g) and those cut from a single strip were treated at the same temperature. Epimysium pieces from right side muscles were later heated at 55 and $70 \pm 2^\circ\text{C}$ and those from the left side were treated at 80 and $95 \pm 2^\circ\text{C}$ with 10 mL of de-ionized water. Heating times employed were as follows: at 55°C 15, 30, 45, 60, 180, 540 and 1440 min; at 70°C 5, 10, 15, 45, 60, 180 and 360 min, and at 80 and 95°C 5, 10, 15, 45 and 60 min. Two raw epimysium pieces (2 x 2½ cm) cut from each strip were stored at -20°C to determine shear stress later. Treatment subsamples were prepared from the adjacent strips.

The remaining epimysium was ground and stored at -20°C for chemical analysis.

3.1.2.2 Properties of epimysium

The frozen epimysium was diced and then repeatedly ground using a Waring commercial blender (Waring Products, Torrington, USA) with alternate freezing in liquid N₂ to obtain fine fibres. Moisture (AOAC 950.46 B, 1990), crude protein (AOAC 981.10, 1990), crude fat (AOAC 960.39, 1990) and collagen (AOAC 990.26, 1990) contents of ground epimysium were determined in duplicate to characterize the raw epimysium.

3.1.2.3 Determination of shear force (SF)

Epimysium samples, both heat treated and raw, pre-cooled to 4° C were cut into 3 strips ½ cm wide, along the direction of the fibres (visible lines on the cleaned epimysium). Strips at room temperature were sheared perpendicular to the fibre direction using TMS-Pro texture system (Food Technology Corp., Sterling, VA, USA) equipped with a Warner-Bratzler shear attachment. Maximum force generated during shearing was recorded as shear force and corrected for the thickness of raw epimysium.

3.1.2.4 Hydrolysis with Pronase

Previous work carried out by Snowden et al. (1978) and Powell et al. (2000) demonstrated that enzymatic digestion of heat treated collagen (free from soluble collagen) with pronase release denatured but insoluble portion of collagen fibres, refer to as amorphous collagen. Based on this observation the following analysis was carried out. After measuring SS, epimysium strips were finely

minced. About 0.8 g was then weighed into 10.4 mL Beckman™ polycarbonate centrifuge bottles (Beckman Instruments, USA). Then it was mixed with 9 mL of 0.5mg/mL pronase solution prepared in 0.12M Tris / 0.1M CaCl₂ buffer (Scott et al. 1981). Samples were placed on a shaker and hydrolysis was carried out for ~16 h at room temperature. At the end, samples were subjected to ultracentrifugation using a Beckman Coulter Optima™ centrifuge with a 70.1Ti rotor and at 80 000 x g/20°C. Clear supernatant was collected and the volume was adjusted to 10 mL with de-ionized water before storage at -20°C. The pellet was discarded. Total protein and Ehrlich chromogen (cross-link) contents of the supernatant were determined.

3.1.2.5 Determination of total protein – BCA protein assay

Hydroxyproline would be the most appropriate indicator of collagen in connective tissue. However, previous work revealed that AOAC 990.26 (1990) colourimetric assay was not sensitive to detect very low levels of hydroxyproline. Therefore, BCA protein assay (Smith et al., 1985) was used to measure protein liberated from epimysium during heating in water, refer to as thermolabile protein (TLP), and protein in the pronase digest refer to as amorphous protein (AMP) as indicators of denatured epimysial proteins

The BCA™ protein assay reagent A (containing sodium carbonate, sodium bicarbonate, sodium tartrate and BCA detection reagent in 0.1N sodium hydroxide) and BCA™ protein assay reagent B (4% cupric sulphate pentahydrate) were mixed in a 100:2 mL ratio, respectively. Thermolabile proteins in epimysium extracts were diluted appropriately before mixing with assay reagents. In a test tube, 2 mL of the assay reagent mix (already prepared) was added to 100 µL of the diluted sample. Contents were immediately mixed on a vortex and then heated at 60°C in a water bath for 30 minutes. Contents were cooled to room temperature before measuring absorbance at 562 nm using UV-VIS spectrophotometer (Genesys 5, Milton & Roy Company, USA). Protein standards were prepared using type B gelatin from bovine skin (Sigma, Canada).

3.1.2.6 SDS-gel electrophoresis

Gel-electrophoresis was carried out to understand the peptide profile of thermolabile proteins. Epimysium extracts preserved at -20°C was thawed to room temperature. Based on protein data from the BCA assay, volumes required to obtain 50 µg of dry protein were calculated. Then exact volumes from the selected treatments were lyophilized in 1 mL micro centrifuge tubes. Then the whole lyophilized sample was mixed with 100 µL of twice concentrated sample buffer (0.1 M Tris at pH 6.8, 2% sodium dodecyl sulphate, 20% glycerol, 0.001% bromophenol blue and 38% de-ionized

water) and 2-mercaptoethanol mixed in 19:1 ratio (v/v), respectively. The mixture was heated for 5 min at 95°C. Resolution was carried out on polyacrylamide gels of 8% resolution and 4% stacking phases. Each well of a gel was loaded with 3 µg of protein. Electrophoresis was carried out at 176 V, in Tris (3.025 g/L) and glycine (14.415 g/L) buffer at pH 8.2. Gels were stained (0.01% coomassie brilliant blue, 50% methanol and 7% acetic acid) for 5 min and then de-stained (10% acetic acid, 7.5% methanol) overnight. Molecular weight marker was purchased from (Bio-Rad Laboratories, Hercules, CA, USA).

3.1.2.7 Determination of Ehrlich chromogen (EC) content

Some of the cross-links of collagen must have broken to liberate α (single) and β (double) chains (as shown on SDS-gels). As such it was considered a useful exercise to determine the ehrlich chromogen (Scott et al., 1981) content in the supernatants from the pronase extractions as enzyme action on (heat-induced) amorphous collagen may also release some cross-linked molecules. The reagents used were 5% dimethyl amino benzaldehyde in 4N perchloric acid/0.01% mercuric chloride. Samples and reagents were mixed in 5.25:1 (v/v) ratio in 2 mL micro tubes. The mixture was centrifuged immediately for 30 s at 12 000 rpm using a 5415 C Eppendorf Centrifuge (Brinkmann Instruments Inc, USA) and at room temperature. Four minutes after mixing the sample and reagent, absorbance was measured at 572 nm using UV-VIS spectrophotometer (Genesys 5, Milton & Roy Company, USA).

3.1.3 Results

The connective tissue sheath around the *longissimus* muscle, regardless of its thickness or macroscopic appearance was considered as epimysium in this study.

Table 3.1.1 shows the basic properties of epimysium. Epimysium from heifers had ~38% protein on a fresh weight basis and collagen contributed to ~88.5% of that. Epimysium from cows had ~43.5% protein and ~91% of that was collagen. Raw cow epimysium (~38.6 N/mm²) showed higher SS values than heifer epimysium (~ 32 N/mm²).

Table 3.1.1 Characterization of epimysium

	Heifer	Cow	P
Protein (g/100g wb)	38.4 ± 0.8	43.5 ± 2.2	0.02
Fat (g/100g wb)	16.4 ± 1.3	9.3 ± 2.0	0.03
Moisture (g/100g wb)	47.2 ± 0.9	47.4 ± 1.7	0.83
Collagen (g/100g wb)	36.4 ± 1.4	40.1 ± 2.1	0.11
Collagen (g/100 g Protein)	88.5 ± 4.0	91.4 ± 4.5	0.01
Shear force(N/mm ²)	32.0 ± 1.6	38.6 ± 3.9	0.02

wb – wet basis; Mean ± SE, n=4.

3.1.3.1 SF data

The changes in SF with the heating time at 55, 70, 80 and 95°C is graphically presented in Fig 3.1.1 (a), (b), (c) and (d), respectively. Interestingly, SF values of mature epimysium were higher than that of heifer epimysium at all temperatures investigated and across all heating times employed. For instance, at the end of 24 h heat treatment at 55 °C, SF values of heifer epimysium were dropped to ~9 N while that of cow epimysium was dropped to only 104 N. SF values were decreased faster (at shorter heating times) at 70°C than at 55°C. As temperature was increased to 80 and 95°C, SF values were decreased further for both cow and heifer epimysium.

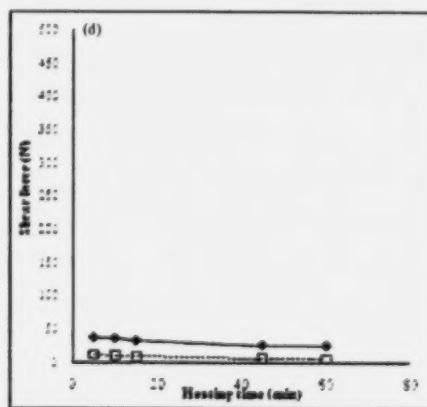
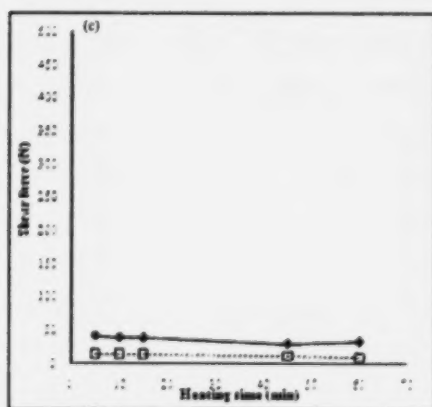
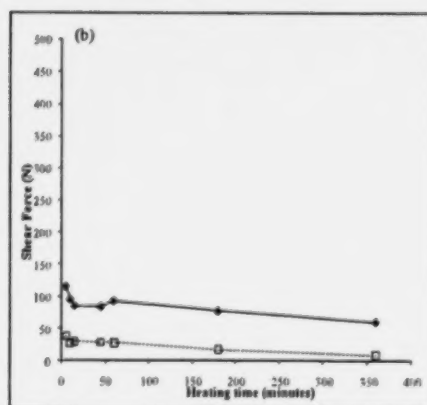
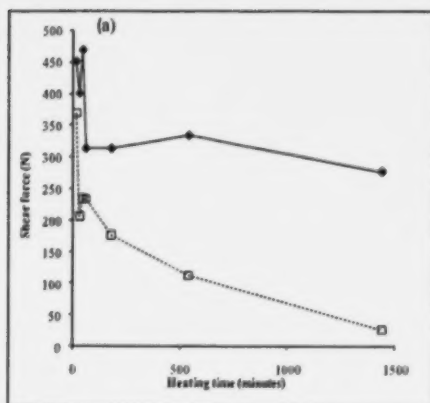


Figure 3.1.1 Changes in epimysial shear force with heating time.

(a) 55°C. (b) 70°C. (c) 80°C. (d) 95°C. Open symbols represent data of heifer epimysium and closed symbols represent data of cow epimysium.

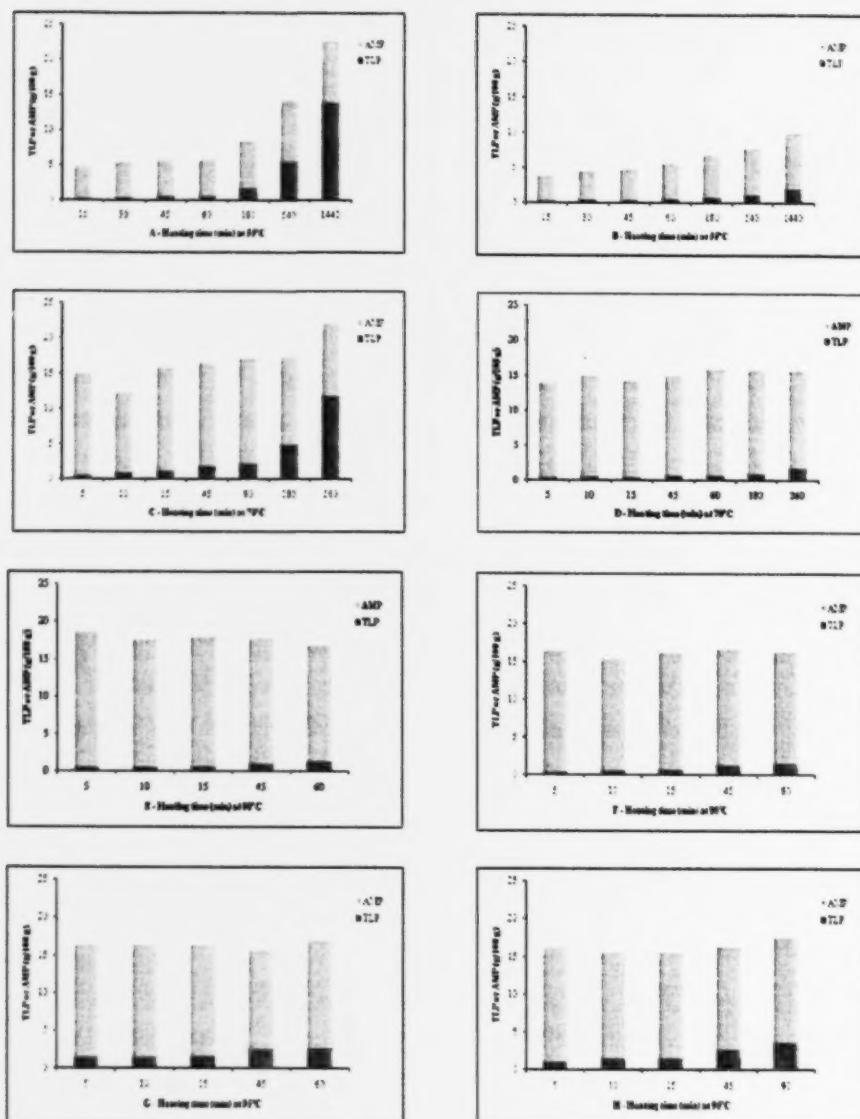


Figure 3.1.2 Changes in the amounts of TLP and AMP of epimysium in relation to temperature and heating time.

A – Heifer epimysium at 55°C. B – Cow epimysium at 55°C. C – Heifer epimysium at 70°C. D – Cow epimysium at 70°C. E – Heifer epimysium at 80°C. F – Cow epimysium at 80°C. G – Heifer epimysium at 95°C. H – Cow epimysium at 95°C.

3.1.3.2 Thermolabile (TLP) and amorphous proteins (AMP)

Thermolabile protein release from the epimysium was significantly influenced by the interaction between maturity*heating time at both 55 ($P<0.05$) and at 70°C ($P<0.05$) and which is graphically presented in Figure 3.1.2 (A-H). At each temperature investigated long heating times increased the amounts of TLP released. However, the amount of TLP released by heifer epimysium after 1440 min heating at 55°C and 360 min heating at 70°C were significantly higher than TLP released at all other treatments. These amounts were several folds higher than that released by cow epimysium under similar conditions of heating. This predicts the thermal resistance of cow epimysial proteins. Also, this indicates that long durations of heating at moderate temperatures can solubilise more collagen from heifer epimysium. Maturity of animals had shown a tendency to have a significant effect on the release of TLP at 55 and 70°C ($P=0.05$), that is heifer epimysium released more TLP than cow epimysium (Figure 3.1.2, A-D). During 60 min heating time, the amounts of TLP released from epimysium were gradually increased with increasing temperature. The amounts of AMP produced were considerably low at 55°C for both cow and heifer epimysium. AMP levels reached the highest values reported just after 5 min heating at 70°C and the slight increase in AMP with heating time was not statistically significant. Heating epimysium at temperatures beyond 70°C did not increase the amounts of AMP formed. There was a tendency to decrease AMP with the long heating times (360 min) at 70°C. When AMP was maintained at constant levels at various heating times and at $\leq 70^\circ\text{C}$, shear force values were continued to decline for both cow and heifer epimysium. Thus, AMP levels should not have an effect on the decreasing shear force values of epimysium.

3.1.3.3 SDS-gel electrophoresis

At 55 °C, TLP of heifer (Figure 3.1.3a) and cow (Figure 3.1.3b) epimysium contained single strands ($\alpha 1$ and $\alpha 2$ chains, >116 kDa) and double strands (2α chains, >200 kDa) of collagen with other unidentified connective tissue proteins. As previously reported, the molecular weight of an α chain of collagen is about 100kDa and that of 2α chains is about 200kDa (Gómez-Guillén et al, 2002; Dreisewerd et al., 2004). Heifer epimysium released single and double strands of collagen starting from 15 min heating at 55 °C but cow epimysium started releasing them only after 30 min heating at 55 °C. Based on the band thickness, heifer epimysium released more double strands than cow epimysium at corresponding heating times. Molecular weights of protein bands produced at 70 °C were similar to those observed at 55 °C for both cow and heifer epimysium, however band thickness increased at 70 °C indicating liberation of more proteins than at 55 °C (gels not shown).

3.1.3.4 EC cross-links

EC (pyrrolic) cross-links in AMP were measured as indicators of thermal damage to epimysium. EC contents were reported as absorbance at 572 nm per unit weight (g) of AMP due to non-availability of EC standards. EC released with the amorphous collagen was neither influenced by the maturity of animals nor by the heating times at both 55 and 70 °C (Figure 3.1.4). Apparently, temperature increase from 55 to 70 °C also had no effect on the amount of 'EC/AMP' released from cow and heifer epimysium. As the amount of EC present in a unit weight (g) of AMP was observed to be a constant it was assumed that EC cross-links were uniformly distributed in amorphous areas of collagen. A preliminary study carried out on EC contents of thermolabile protein extracts from epimysial heating at 55 and 70 °C had shown consistent but almost negligible absorbance values for all treatment combinations. Therefore, analysis of EC in thermolabile protein extracts was abandoned.

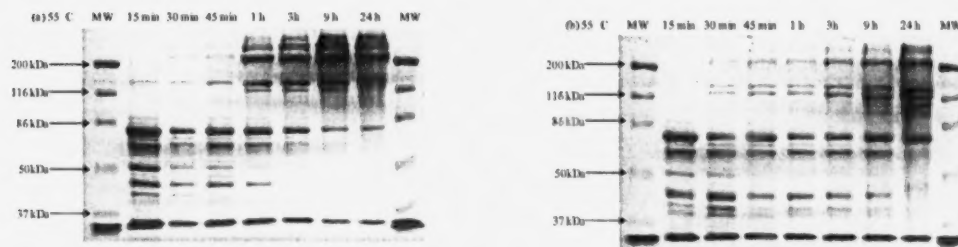


Figure 3.1.3 SDS-PAGE showing peptides released during heating of epimysium at 55°C.

Figure 3.1.3 SDS-PAGE showing peptides released during heating of epimysium at 55°C.

Molecular weight markers (MW) are placed at the two ends of the gels. (a) Peptide profile of TLP from heifer epimysium at 55°C. (b) Peptide profile of TLP from cow epimysium at 55°C. Peptide bands at 116 and 200 kDa are single ($\alpha 1$ and $\alpha 2$) and double (β) chains of collagen.

3.1.4 Conclusions

Collagen contributes to about 90% of epimysial proteins. Physicochemical properties of epimysium should, therefore, be governed by the properties of collagen. Epimysial properties assessed, except for shear stress, did not change with the animal maturity, heifers vs. cow. The conversion of collagen from a native state to an amorphous state (extracted by pronase) is not related animal maturity and changes in shear force. Shear force values of heifer epimysium can be significantly decreased by heating at 70°C and as heating time increased. Shear force values of cow epimysium can be decreased by increasing both temperature (>70°C) and heating time

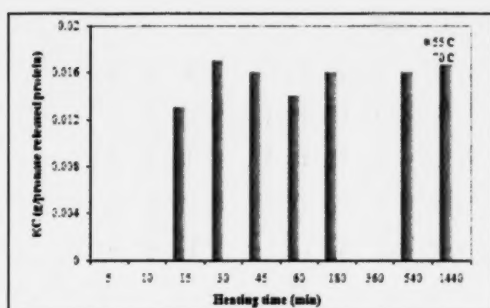


Figure 3.1.4 Changes in EC content of AMP during heating at 55 and 70°C.

Acknowledgements

We gratefully acknowledge the funding provided for this project by the Value-Added Beef Program through the Saskatchewan Beef Development Fund and material provided by the Canadian Cattlemen's Association.

3.2 Evaluate the efficacy of chemical treatments on properties of connective tissue/meat cuts from mature animals

Status: This article contains excerpts from the PhD Thesis of Anula Perera.

(http://library2.usask.ca/theses/available/etd-03252009-54013/unrestricted/Anula_Perera_Thesis.pdf).

3.2.1 Introduction

The contribution of connective tissue to beef toughness was extensively investigated (Sifre et al., 2005; Purslow, 2005; Lepetit, 2007). Perimysial and endomysial collagen content and collagen fibre diameter were reported to correlate with beef toughness (Light et al., 1985). Meat tenderization treatments are generally applied onto the meat matrix in which connective tissue and muscle fibres are interconnected together. As a result, the effect of tenderizing agents on the contributors to toughness and particularly on connective tissue itself is not elucidated.

Alkalis are not widely used as meat tenderizers except for some attempts to use sodium bi carbonate (Sheard and Tali, 2004) and ammonium hydroxide (Hamling and Calkins, 2008). Sodium hydroxide

is widely used in the production of gelatine from animal sources but not as a meat tenderizer. Also, there is evidence that toughness (shear stress) of epimysium from youthful cattle (heifers) can be substantially decreased through aqueous heating but epimysium from mature cattle (cows) remains tougher before and after aqueous heating [Perera, 2009]. In this study, cow epimysium was used as a model to assess the potential to utilize sodium hydroxide and ammonium hydroxide as connective tissue tenderizers and to understand the impact of alkali on connective tissue properties.

To reduce the connective tissue driven toughness, beef was marinated with organic acids such as acetic, lactic and citric (Arganosa and Marriott, 1989; Aktas and Kaya, 2001; Berge et al., 2001). The application of acids to tenderize meat might be linked to their ability to solubilize collagen (Bowes et al., 1955; Kruggel, et al., 1970; Whiting and Strange, 1990; Meyer, 2003). Acetic acid (CH_3COOH) *per se* was often used for the isolation of collagen from tissues (Maekawa et al, 1970; Rhodes and Miller, 1978; Nagai and Suzuki, 2000).

3.2.2 Methodology

3.2.2.1 Epimysium sample preparation

Six *longissimus* muscles from cows of utility grade were purchased and stored at 4 °C. On the 10th day post-mortem, dorsal epimysium was excised and stored at -20 °C. Prior to use, visible fat and muscle fibres were removed and the epimysium was cut into two strips (width 2 cm) along the length. From the strips, pieces of 2x2.5 cm were prepared. Treatments were applied in a split-plot design, temperature as the main plot and concentration of alkali or acid and pre-equilibration time as sub-plots. This experiment was repeated with epimysium from three different animals.

Epimysium pieces were pre-equilibrated (0, 90, 180 min) in 10 mL of aqueous sodium hydroxide (0.01, 0.025, 0.05 M; pH 12.0-12.7) and aqueous ammonium hydroxide (0.1, 0.25, 0.5 M; pH 11.1-11.4) prior to heating at 55 °C and 70 °C for 15 min. As a control, epimysium pieces from the same animals were heated in de-ionized water (10 mL) for 15 min at 70 °C. Acid treatments were applied in a similar manner to alkalis. Each epimysium piece within a temperature treatment was immersed in 10 mL of HCl or CH_3COOH (0.1, 0.25 and 0.5M) concentrations. The pH values of HCl and CH_3COOH solutions were in the ranges of 1.10-1.54 and 2.99-3.21, respectively.

3.2.2.2 Protein determination

Protein released from epimysium was measured using the bicinchoninic acid (BCA) assay procedure, as already explained in activity 2.

3.2.2.3 Epimysial shear stress

Epimysial shear stress before and after heating with alkali was determined as explained in activity 2. The maximum shear stress generated was expressed as the force applied on a unit cross sectional area of sample and also corrected for raw thickness (N/mm^2).

3.2.2.4 Thermal denaturation temperatures

Thermal denaturation temperatures of epimysial proteins were determined. Briefly, raw epimysium from 6 cows was first cut into small pieces and then ground after freezing in liquid N_2 . About 0.5 g of ground epimysium was suspended in 10 mL of de-ionized water (control), 0.25 M NH_4OH and 0.025 M NaOH. Samples were equilibrated overnight at 4 °C. Excess liquid was drained out and epimysium samples were briefly dried on filter paper. Samples (7-8 mg) were weighed into coated aluminium pans and hermetically sealed with lids. Pans were heated from 20 to 140 °C using a differential scanning calorimeter (DSC Q 2000, TA Instruments, USA) at a rate of 5 °C/min. Sealed, empty pans were used as the reference. Data analysis was carried out using curve integration software (TA Universal Analysis 2000).

3.2.2.5 Infrared spectroscopy

IR spectra of amide bands of epimysial proteins were determined. Epimysium pieces (2x2.5 cm) were suspended in 10 mL of de-ionized water (control) and 0.5 M NH_4OH (180 min pre-equilibration). In a pre-heated water bath, the internal temperatures of samples were brought to 70 °C and heating continued for 15 min. Samples were stored at -20 °C. Selected samples were sectioned, placed in plastic moulds and filled with embedding compound OCT cryomatrix® (Shandon Company, USA). The preparation was frozen in liquid nitrogen and stored at -20 °C. Serial sections (6 μm) were cut from cryo-preserved samples using a cryo-microtome. Sections were placed on reflective-coated Low-e® microscope slides (Kevley Technologies, USA) for spectroscopic analysis. FTIR imaging was conducted using 01B1-1 Mid IR beam line ($800\text{-}2000\text{ cm}^{-1}$) at the Canadian Light Source. Spectroscopic mapping was performed in reflection mode with diffraction-limited spatial resolution. For each pixel, 64 scans, with a 4 cm^{-1} spectral resolution, were co-added. Data analysis was conducted using OPUS software (Bruker Optics, Germany).

3.2.3 Results

Moisture, protein and fat levels of the epimysium samples used are presented in Table 3.2.1. Raw epimysium samples had shear stress values of $38 \pm 7\text{ N}/\text{mm}^2$.

Table 3.2.1 Properties of cow epimysium (N = 18).

Property	Mean $\pm$ SD
Moisture (g/100g wb)	55.2 $\pm$ 1.7
Protein (g/ 100g wb)	45.6 $\pm$ 3.7
Fat (g/100g wb)	3.9 $\pm$ 1.6
Shear stress (N/mm ²)	38.4 $\pm$ 6.8

3.2.3.1 Protein released

After alkali treatments, the maximum amount of protein released was <2% of the weight of epimysium or <5% of epimysial proteins. Following 180 min pre-equilibration in sodium hydroxide (0.01-0.025 M) and subsequent heating to 55°C, single (α) and double strands (β) of collagen were liberated (Figure 3.2.1a). As the concentration of sodium hydroxide (0.05 M) and temperature (70 °C) were increased, collagen peptides were hydrolysed. Ammonium hydroxide also had liberated single and double strands of collagen at the two temperatures investigated (Figure 3.2.1b). Some hydrolysis of collagen peptides was noted after 0.5 M ammonium hydroxide treatment (180 min pre-equilibration) followed by heating to 70 °C. The amounts of protein released from epimysium subsequent to acid treatments are presented in Figure 3.2.2 (a-d). Protein release was influenced by the increasing concentration of acid, pre-equilibration time in acid before heating and mostly by the temperature of heating. As expected, pre-equilibration treatment of 90 and 180 min in 0.5M HCl had significantly solubilised the epimysial proteins (Figure 3.2.2b). Acetic acid at the highest concentration (0.5M) had solubilised less than 3g proteins/100g epimysium (Figure 3.2.2d).

3.2.3.2 Shear stress

Shear stress values of epimysium (raw 38 N/mm²) was decreased to ~25-29 N/ mm² as samples were pre-equilibrated (0-180 min) in sodium hydroxide (0.01-0.025 M) and subsequently heated to 55°C (Figure 3.2.3a and 3.2.3b). The decrease in shear stress is attributed to the effect of temperature (55°C) because samples heated in water had reached similar values (~23 N/mm²). As the concentration of sodium hydroxide was increased to 0.05 M, shear stress was further decreased by 10 N/mm². Ammonium hydroxide concentration (0.1-0.5 M) and pre-equilibration (0-180 min) in it did not decrease shear stress (~39 N/mm²) at 55°C (Figure 3.2.3c and 3.2.3d). The effect of temperature (55°C) in decreasing shear stress was eliminated by ammonium hydroxide treatment because samples heated in water had reached 24 N/mm².

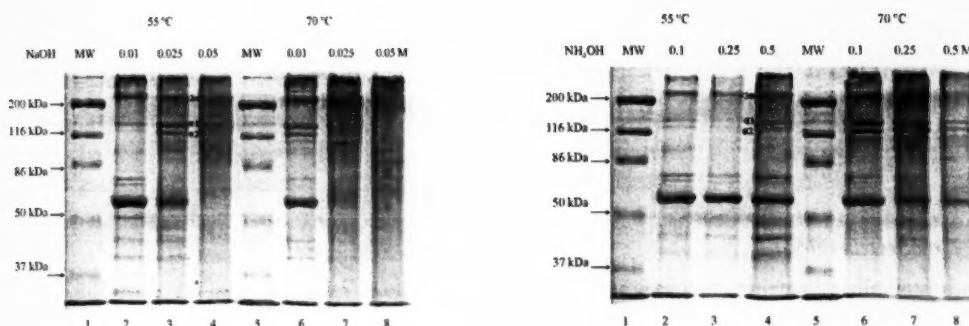


Figure 3.2.1 Peptides released from epimysium following alkali treatments.

Samples were pre-equilibrated for 180 min in alkali and heated at 55 and 70 °C for 15 min. (a) Lanes 2, 3, 4 (55°C) and lanes 6, 7, 8 (70°C) show sodium hydroxide concentrations 0.01, 0.025 and 0.05 M, respectively. (b) Lanes 2, 3, 4 (55°C) and also lanes 6, 7, 8 (70°C) show ammonium hydroxide concentrations 0.1, 0.25 and 0.5 M, respectively. Lane 1 and 5 are molecular weight standards (kDa). α1 and α2 represent single strands of collagen and β represents double strands of collagen.

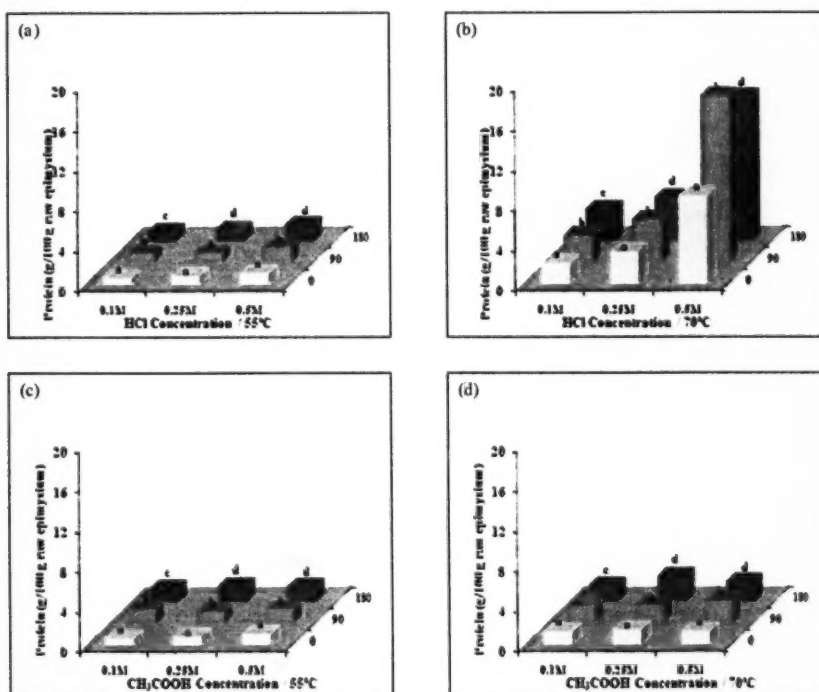


Figure 3.2.2 Protein release data of epimysium after acid treatments.

(a) Heating in HCl at 55°C. (b) Heating in HCl at 70°C. (c) Heating in CH₃COOH at 55°C. (d) Heating in CH₃COOH at 7°C.

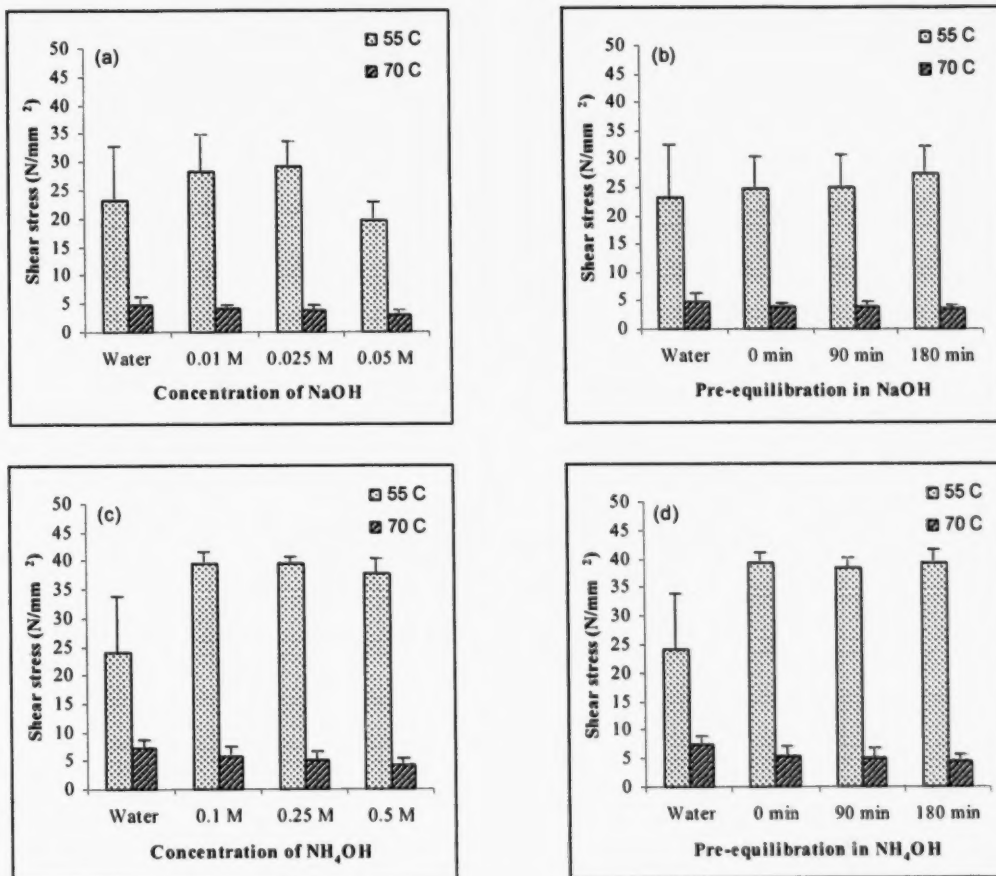


Figure 3.2.3 Changes in epimysial shear stress following alkali treatments.

(a) Effect of the concentration of ammonium hydroxide and temperature. (b) Effect of the pre-equilibration time in ammonium hydroxide and temperature. (c) Effect of the concentration of sodium hydroxide and temperature. (d) Effect of the pre-equilibration time in sodium hydroxide and temperature.

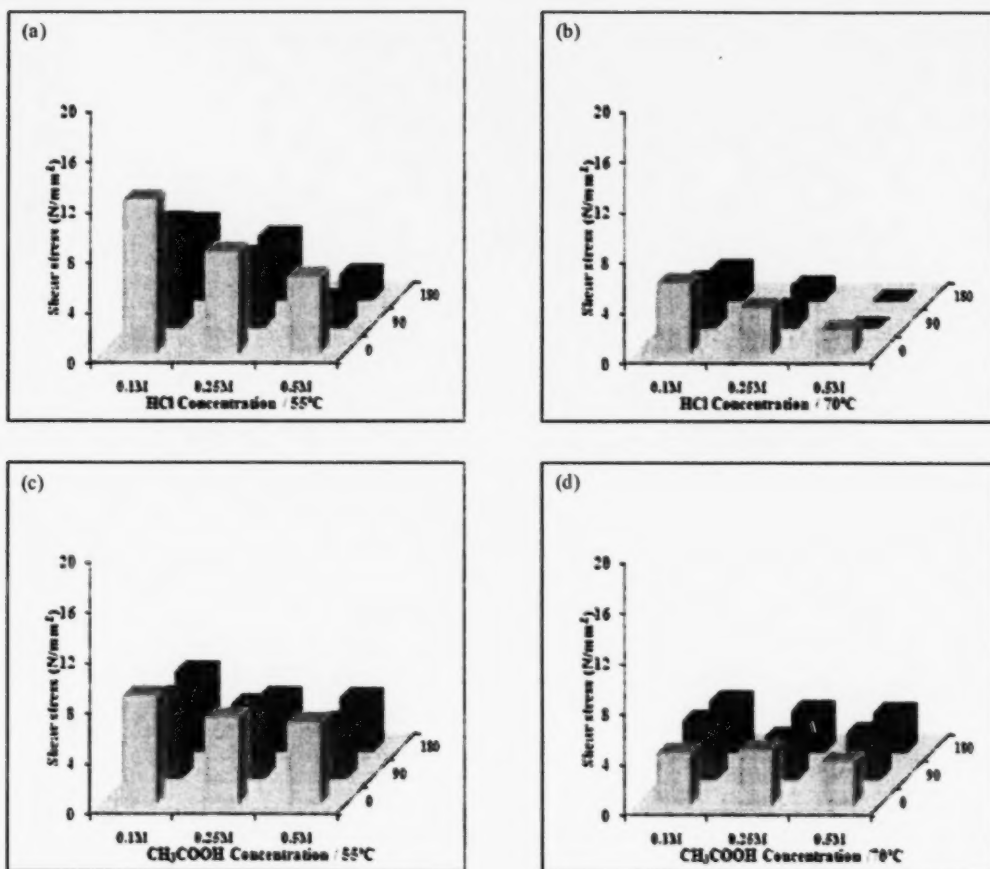


Figure 3.2.4 Effect of acid treatments on epimysial shear stress.

(a) Effect of the concentration of HCl and pre-equilibration time at 55°C. (b) Effect of the concentration of HCl and pre-equilibration time at 70°C. (c) Effect of the concentration of CH₃COOH and pre-equilibration time at 55°C. (d) Effect of the concentration of CH₃COOH and pre-equilibration time at 70°C.

Heating at 70°C had a significant effect on epimysial shear stress reduction. Shear stress of sodium hydroxide and ammonium hydroxide treated epimysium was decreased to 3-6 N mm^{-2} after heating at 70°C and which was similar to that heated in water ($\sim 5 \text{ N/mm}^2$). The epimysium stabilizing effect of ammonium hydroxide was eliminated at 70°C.

Shear stress values of acid treated epimysium are presented in Figure 3.2.4 (a-d). The increasing concentration of acid, pre-equilibration time in acid and temperature of heating had significantly contributed to the decreased shear stress values. The effect of HCl is more prominent at 70°C where at 0.25M concentration shear stress decreased to $< 3 \text{ N/mm}^2$ and at 0.5M concentration of acid epimysium was mostly solubilized and shear stress could not measure (Figure 3.2.4b). At 0.25 and 0.5M concentrations and at 70°C, acetic acid was effective in decreasing epimysial shear stress $< 5 \text{ N/mm}^2$.

3.2.3.3 Thermal denaturation temperature

Epimysium samples saturated in water prior to heating had a thermal denaturation temperature of $\sim 63^\circ\text{C}$ (Table 3.2.2). At the concentration used (0.025 M), sodium hydroxide did not change the melting temperature of epimysial proteins ($\sim 64^\circ\text{C}$). Following ammonium hydroxide treatment (0.25M), denaturation temperature was significantly increased to $\sim 66^\circ\text{C}$ with a narrower and a deeper denaturation curve compared to other treatments. This observation supports the hypothesis that ammonium hydroxide has a structure stabilization effect on collagen because $\sim 91\%$ of the epimysial proteins consisted of collagen (Perera, 2009).

Table 3.2.2 Melting temperatures of epimysial proteins after aqueous and alkali heating. (Mean $\pm$ SE)

	Melting temperature peak ($^\circ\text{C}$)	Onset temperature ($^\circ\text{C}$)
Water	63.0 ± 0.5	53.3 ± 0.6
0.025 M NaOH	64.0 ± 0.3	55.9 ± 0.6
0.25 M NH_4OH	66.2 ± 0.1	55.9 ± 0.5
P	0.05	0.05
LSD	2.00	2.18

Heating carried out at $5^\circ\text{C}/\text{min}$ from 20-140°C. Each treatment had 6 replicates and three sub samples.

3.2.3.4 Infrared spectrum

After aqueous heating of epimysium (from animals < 30 months and 3-5 years) at $70\text{-}95^\circ\text{C}$, 14-16 g amorphous proteins in 100 g epimysium (or $\sim 30\%$ on a protein basis) was extracted with pronase (Perera, 2009) indicating helix $\rightarrow$ coil transition. However, aqueous heating at 70°C (15 min) did not introduce changes to the typical amide bands I ($1675\text{-}1658 \text{ cm}^{-1}$) and II (1550 cm^{-1}) of epimysial proteins (Figure 3.2.5a). Therefore, C=O and N-H bond stretching (Pelpis et al., 1996) might not have happened in collagen peptides during heating at 70°C for 15 min, even though some of the epimysial

collagen converted to an amorphous state under similar conditions of heating (Perera, 2009) or else, significant recoiling of collagen (coil→helix transition) (Hörmann and Schlebusch, 1971) might have happened during cooling. A new peak emerged ($1640\text{--}1620\text{ cm}^{-1}$) on the shoulder of the amide I band of ammonium hydroxide treated and subsequently heated epimysium (Figure 3.2.5b). This band represents random coils (Martinez and Millhauser, 1995) and also β sheets (Byler and Susi, 1986). Because glycine contributes to $\frac{1}{3}$ of amino acids of major epimysial protein collagen (Estoe (1967) it is thought this new peak represents random coils (Smith et al., 1994). Perhaps, ammonium hydroxide has prevented or decreased the coil→helix transition of thermally denatured collagen.

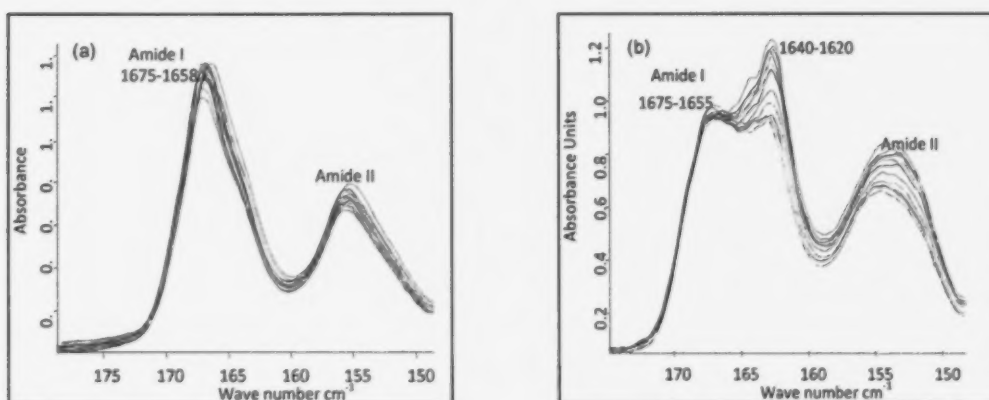


Figure 3.2.5 IR spectra of epimysium.

(a) Heated to $70\text{ }^{\circ}\text{C}$ with de-ionized water with no pre-equilibration. Amide bands I ($1675\text{--}1658\text{ cm}^{-1}$) and II (1550 cm^{-1}) typical to proteins are observed despite the exposure to high temperature. (b) Pre-equilibrated for 180 min in $0.25\text{ M NH}_4\text{OH}$ and heated to $70\text{ }^{\circ}\text{C}$ for 15 min. On the shoulder of the characteristic amide I band ($1675\text{--}1655\text{ cm}^{-1}$) a new peak emerged ($1640\text{--}1620\text{ cm}^{-1}$).

3.2.4 Conclusion

Sodium hydroxide (0.05 M) tenderizes connective tissue more than water at 55°C but at 70°C , water is an equally good tenderizer as alkali. Ammonium hydroxide ($0.1\text{--}0.5\text{ M}$) had no useful tenderizing effect on connective tissues. Instead, ammonium hydroxide had a collagen stabilization effect and raised the melting temperature of collagen. Acids tenderize epimysium through solubilising proteins and the effect of acid is more prominent at 70°C than at 55°C . Pre-equilibration of epimysium in acids prior to heating helps to further decrease shear stress more than heating in acid without a pre-equilibration treatment.

Acknowledgement

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ACTIVITY 4: COMBINED MICROWAVE AND CONVECTION DRYING AND TEXTUTAL CHARACTERISTICS OF BEEF JERKY

Status: This article contains excerpts from the MSc Thesis of Victoria Thiagarajan. (<http://library2.usask.ca/theses/available/etd-10102008-150255/unrestricted/Thiagarajan-Thesis.pdf>)

4.1 Introduction

Jerky is a popular meat snack, rich in protein. The low caloric value and low fat content made it acceptable to wide range of consumers. The name jerky was derived from the Spanish word 'charqui', meaning dried meat. Jerky is made by slicing the meat into strips and dry or by grinding the meat and dry. The processing of jerky involves salting and drying to improve its shelf life. Due to recent food poisoning outbreaks, the processing procedures for jerky had been regularized by the United States Department of Agriculture (USDA, 2003). As per the present recommendation, jerky having moisture to protein ratio of 0.75:1 and water activity not more than 0.85 is said to be safe for human consumption. Also, it is recommended that the product temperature should reach 71°C during processing (USDA, 2003). The commonly reported methods of jerky processing include drying in a smoke-house and home dehydration. The American Association of Meat Processors (2004) reported that this traditional method takes 6-10 h at 60°C. Reducing the processing time would certainly lead to the reduction in the product cost.

It is reported for several other food products that it is 75 % more energy efficient to utilize electromagnetic energy for the drying process (Quenzer and Burns, 1981). There are advantages in utilizing microwave energy over conventional types in terms of energy savings, reduced processing time, enhanced quality attributes and cost effectiveness. Application of any new innovative, energy efficient technology has not been experimented for jerky processing yet. The present study attempted to explore the possibilities of using different drying methods for jerky processing.

4.2 Methodology

4.2.1 Sample preparation

Beef *biceps femoris* muscles were used in this study. The excess fat on meat was trimmed off and stored at -30°C until used. Meat was thawed at 4°C and ground using a meat grinder fitted with a kidney plate. The coarsely ground meat were blended in a vacuum tumbler for 2 minutes. Again the meat was ground twice using a blade having 1/8 inch slots. The other ingredients used (Table 4.1) were mixed with meat in a lower speed using an industrial blender for 105 sec. To achieve the desired

pH levels of 5.65, 5.30 and 5.15, glucono delta lactone (GDL) was added at the percentage of 0, 0.5 and 1, respectively, while blending. The mixture was then fed into the industrial food extruder to get 6 mm thick samples, which were then vacuum packed and stored in -30°C for further processing.

Table 4.1. Formulations of beef jerky samples

Treatment	Meat (g)	GDL*(g)	Salt (g)	Sugar (g)	Prague (g)	Na Ery** (g)	Water (g)
P1S1	2817.92	0	21.58	60	9	1.5	90
P1S2	2787.92	0	51.58	60	9	1.5	90
P1S3	2757.92	0	81.58	60	9	1.5	90
P2S1	2802.92	15	21.58	60	9	1.5	90
P2S2	2772.92	15	51.58	60	9	1.5	90
P2S3	2742.92	15	81.58	60	9	1.5	90
P3S1	2757.92	30	21.58	60	9	1.5	90
P3S2	2757.92	30	51.58	60	9	1.5	90
P3S3	2727.92	30	81.58	60	9	1.5	90

where *Glucono delta lactone and **Sodium Erythorbate

4.2.2 Combined microwave-convection drying

Drying experiments were carried out in a laboratory scale modified microwave oven which was designed to dry/heat with microwave, convection and a combination of convective and microwave energy. The frozen samples were thawed for 5 h at room temperature (23°C) prior to drying. Each test was conducted with a 25 g sample. A setting of 295 Watts, pulse microwave (14s ON and 7s OFF), 70°C air temperature and 1.45 m/s air flow rate were used to dry the samples to 0.33% moisture content on a dry basis.

4.2.3 Forced air thin layer drying

For this experiment, 2% salt content, 2% sugar, 0.3% prague, 0.05% sodium erythorbate and 3% water were mixed with 3 kg of meat and the samples were prepared using the method previously described.

The laboratory scale forced air thin layer cross flow drying unit was used to dry the samples. This system consists of an air conditioning unit, a drying chamber, a vane axial circulating fan and a connecting duct system. The air conditioning unit has an operating temperature range of 17-200°C and relative humidity range of 2-98%. The tests were conducted at 40 and 15 % relative humidity and

1 and 1.45 m/s airflow rate with a constant air temperature of 80°C. Samples were placed in the drying trays and product weight change was monitored online using Labview 6.0 (National Instruments, Austin, TX).

4.2.4 Smoke house processing

A batch smoke oven was used to process jerky. The raw samples were placed on the trays of the scrolling truck with screen size of 42" x 42". The drying trials were done with two replications.

4.2.5 Quality determination

Moisture content: Initial and final moisture content of the sample was measured as per ASAE S353 (2003) method.

Water activity measurement (a_w): Water activity of the dried samples was measured using the Aqua lab water activity meter (Model CX2, Decagon Devices Inc., Washington, USA). The slices were cut into fine pieces for taking the measurements. Each sample was measured in triplicate.

Shrinkage loss: Initial and final volume has been measured and shrinkage coefficient was calculated. Samples were tested three times for each treatment.

pH: pH of the fresh and dried samples were measured using the pH meter (Accumet-Model 15, Fisher Scientific). It was repeated for three times for each treatment.

Color: Color of the jerky before and after drying was measured using Hunterlab Color Analyzer-Labscan-2 (Hunter Associates Laboratory, Inc. Virginia, USA).

Texture profile analysis: A Universal Texture Analyser TA.XT2 (shown in Figure 3.3) was used for texture profile analysis. Cubes of dried meat samples, 10 x 5 x 10 mm, were placed parallel to the compression plate surface and compressed twice to 50% of their original height, keeping the time between successive compressions to zero. A 25 kg load cell was used to compress the samples. Force-time curves were recorded at a crosshead speed of 5 mm/s.

Puncture test: A puncture test was performed using a TA Texture Analyzer with a TA-53 3 mm stainless steel probe. The 12.5 mm jerky strips were laid over a 10mm hole and the 3mm diameter

probe was punched 10 mm into the sample at a crosshead speed of 0.5mm/sec. A 25 kg load cell was used. The probe was programmed to punch completely through the jerky each time.

Tensile test: Tensile strength and energy necessary to pull the jerky apart was measured using an Instron universal machine (Model 1011, Instron Corp., Canton, MA). Initial grip separation was set to 50 mm and crosshead speed set to 60 mm/sec. A 25 kg load cell was used to pull the strip off. The tensile force, energy and elongation were recorded for each sample.

4.3 Results

4.3.1 Microwave and convection drying

4.3.1.1 Extrinsic properties

The drying curves shown in Figures 4.1, 4.2 and 4.3 illustrate the different trends followed by the samples with different pH values while drying. Overall, it was found that lowering the pH increased the drying rate, which in turn reduced the time taken to dry the samples. Moisture removal at first 2-3 minutes of drying was found to be high in all the treatments. Due to the high heating rate of microwaves, the jerky samples with 1% salt followed a high constant rate of moisture removal throughout the drying period. The samples with lower pH had shown higher moisture removal rate and hence they dried more rapidly than the others.

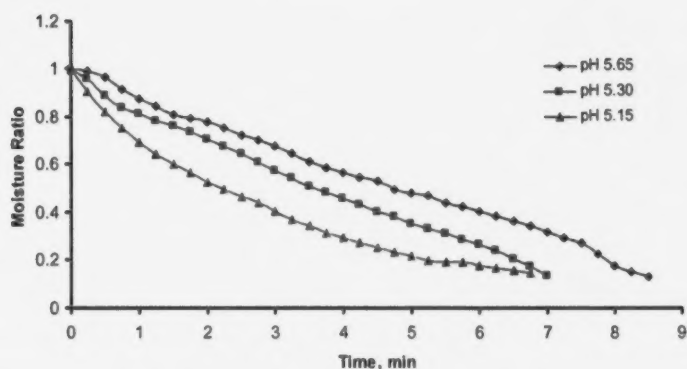


Figure 4.1 Drying curves of jerky samples having 1% salt content.

Combined microwave-convection drying was used at pH levels 5.65, 5.30 and 5.15.

For jerky with 2% salt content, the moisture removal rate was high at the beginning and then due to the interaction of salt and microwaves, the drying rate became more rapid (Figure 4.2). Due to the

combined effect of salt and pH, the samples have shown higher moisture removal at the beginning and a high constant moisture removal rate was followed until the end of the drying process. Samples with 3% salt content also behaved similarly (Figure 4.3) to those with 2% salt while drying. In the case of samples with 3% salt, the drying was even faster compared to 2% salt content samples, likely due to the higher dielectric loss factor contributed from the addition of 3% salt.

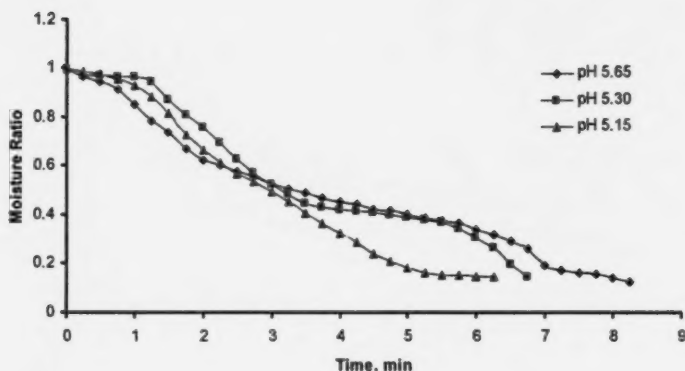


Figure 4.2 Effect of pH on microwave-convection drying of jerky at 2% salt.

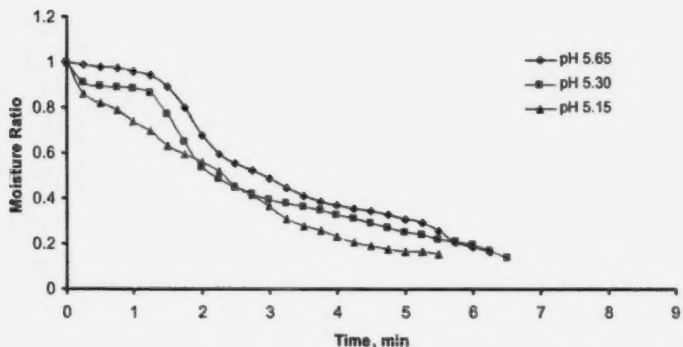


Figure 4.3 Effect of pH on microwave-convection drying of jerky at 3% salt.

Figure 4.4 shows the drying rate curves of the samples with different salt content at pH 5.65 and revealed the effect of salt content on drying. Samples with 1% salt showed a higher moisture removal at the beginning of drying. At this period the surface water was evaporated due to the higher heating

capability of microwave energy. Later, drying was carried out at a lower but constant rate till the end. Samples with 2% salt content have followed a similar trend as samples with 1% salt content. But, for the samples with 3% salt content initial moisture removal was slow and then the moisture content was dropped sharply due to the higher absorption of microwaves and higher internal heat created.

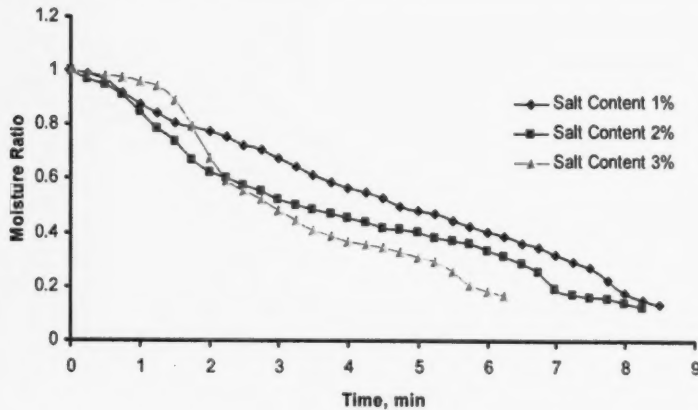


Figure 4.4 Effect of salt content on microwave-convection drying of jerky at pH 5.65.

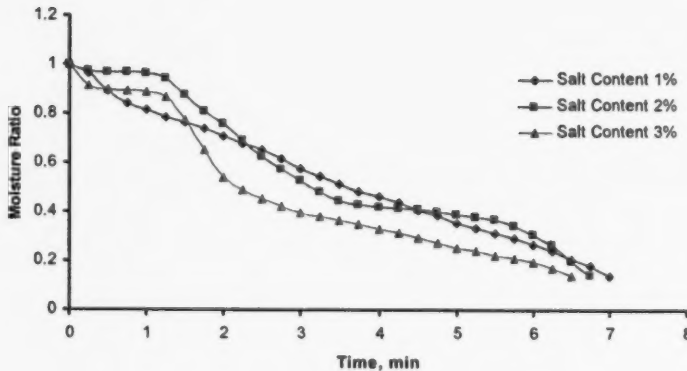


Figure 4.5 Effect of salt content on microwave-convection drying of jerky at pH 5.30.

Drying curves of the samples dried with different salt contents at pH 5.30 are shown in Figure 4.5. For the samples with 1% salt content, drying rate was constant throughout the process except at the

beginning. Initial moisture removal rate were found to be higher compared to the rest of the drying period. Samples with 2% behaved similarly. The drying rate was high at the beginning of the drying, and then the drying rates were dropped sharply to maintain a constant rate. However, the 3% salt content samples had higher drying rates throughout drying than the others.

Drying rate curves shown in Figure 4.6 illustrate the effect of salt content at pH 5.15 on beef jerky. All the samples behaved similarly, except 2% salt content sample had a slow drying stage at the beginning then, the drying rate has reached its maximum after 2 min and then dropped to follow a constant drying rate. The drying rate of the samples at pH 5.15 was significantly higher than higher pH samples, due to the combined ionic effects of salt and pH and the increasing heating in the presence of microwave energy.

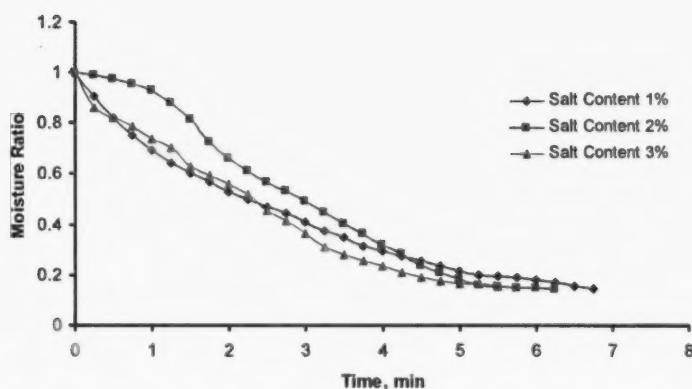


Figure 4.6 Effect of salt content on microwave-convection drying of jerky at pH 5.15.

4.3.1.2 Physicochemical characteristics

Shrinkage: The effects of pH and salt content on shrinkage loss, weight loss and water activity of jerky are shown in Table 4.2. Shrinkage and weight loss were significantly influenced at constant moisture content. The lower pH samples had higher shrinkage and weight loss. As the moisture removal rate was higher in lower pH samples compared to higher pH samples, more dimensional deformation occurred in samples with lower pH. The dimensional change varied from 32.14 to 49.84 % when pH of the samples was changed from 5.65 to 5.15 at 1% salt content. The shrinkage value observed in jerky with 2% salt content was increased ($p < 0.05$) from 37.77% to 54.95% with a

reduction in the pH value from 5.65 to 5.15, which lead to a significant increase in weight loss. pH and salt content have significantly influenced the shrinkage and weight loss of beef jerky dried in combined microwave-convection drying. Lowering pH and increasing salt increased the shrinkage and weight loss. Samples with 3% salt at pH 5.15 were showed the highest shrinkage and weight loss. Whereas, samples with 1% salt at pH 5.65 had the lowest losses in terms of weight and volume.

Water activity: Water activity of samples with different pH values and at 2 and 3% salt content were significantly different ($p < 0.05$) from each other, though there was no difference found between samples with pH 5.65 and 5.30 at 1% salt content (Table 4.2). However, the water activities of samples with 1% salt content and having pH 5.65 and 5.30 fell out of the safe range (8.5) recommended by USDA (2003). The products reached temperatures of 81.4 and 88.2°C. The sample water activity varied from 0.82 to 0.77, when the pH was reduced from 5.65 to 5.15. Lower pH samples had lower water activities. Water activity was significantly influenced by salt content. The water activity was reduced to 0.75 from 0.85 by the addition of 3% salt at pH 5.30. At pH 5.15 the effects of addition of salt on water activity were not significant within the samples having 1 and 2 % salt. However, the samples with 3% salt have shown significant reduction in water activity than other samples at pH 5.15.

Table 4.2 Physical-chemical characteristics of beef jerky dried under combined microwave-convection drying.

pH	Salt Content (%)	Shrinkage (%)	Weight loss (%)	Water activity
5.65	1	32.14 ± 1.75 h	56.54 ± 0.00 f	0.86 ± 0.00 a
	2	37.77 ± 0.29 g	57.10 ± 0.10 e f	0.82 ± 0.01 b
	3	48.32 ± 0.57 e	58.15 ± 0.28 d	0.80 ± 0.00 c
5.30	1	42.34 ± 0.75 f	57.59 ± 0.36 d e	0.85 ± 0.01 a
	2	52.11 ± 0.54 c	59.38 ± 0.22 c	0.78 ± 0.00 c d
	3	53.33 ± 0.41 c	60.60 ± 0.56 a	0.75 ± 0.01 e
5.15	1	49.84 ± 0.85 d	59.10 ± 0.34 c	0.79 ± 0.00 c d
	2	54.95 ± 0.61 b	60.36 ± 0.30 b	0.77 ± 0.01 d
	3	60.44 ± 1.89 a	62.57 ± 0.51 a	0.68 ± 0.00 f

Means in the same column with different letters are significantly different ($p < 0.05$).

Color: The color data of jerky samples show that the lightness value has increased when the sample pH was altered from 5.65 to 5.30 and 5.15. The redness values were measured for beef jerky dried in combine microwave-convection drying as 12.48 to 21.44. Microwave-convection drying has increased the redness of the sample; this is based on the initial redness value of the raw jerky samples. The b* value was in the range of 7.45 to 15.02.

Puncture test data: The puncture forces measured were in the range of 30.87 N to 51.82 N. These values are considerably lower than the reported puncture values by other researchers. Lee and Kang (2003) have reported the puncture force required by intact ostrich jerky (24% wb) as 148.3 N. Also the puncture force of beef jerky commercially sold in Korea, was 128.9 N (Lee and Kang, 2003). The effect of pH on puncture force was not statistically ($P < 0.05$) significant.

Tensile properties: Tensile forces of dried jerky samples were within the range of 31.10 N to 91.40 N. The samples dried in combined microwave-convection dryer had lower tensile force, energy and higher elongation than previous reports. The samples with the lowest pH required more force to stretch the samples, which indicates that the stiffness of the product was increased with the acidity level. At 1.28% salt content, the difference in tensile force between higher pH levels was not significant. The samples having pH 5.30 and 1.28% salt content had the highest elongation. Also, this treatment samples required less tensile force. The tensile energy was also significantly affected by pH change. The pH 5.15 samples had more tensile force with less displacement, which indicates the sample have lower elasticity than other two treatments. The energy required to pull the samples were found to differ significantly. At 3.28% salt content, lower pH samples possessed higher tensile force and lower displacement value. This indicates that pH 5.15 samples were tougher than higher pH samples. Hardening of high acid products can be explained by the faster moisture removal taking place during drying. The tensile properties of the beef jerky were significantly influenced by the amount of salt added. Tensile force was observed as 31.10 N with elongation of 4.37mm for 1.28% salt and 62.70 N with 3.35 mm of elongation for 3.28% salt. Samples with 2.28% salt required higher energy than the samples with 1.28 and 3.28% salt to stretch the jerky samples.

4.3.2 Thin layer drying

4.3.2.1 Extrinsic properties

The drying rate was significantly increased when the relative humidity of the air was reduced from 40% to 15. The effect of relative humidity on drying rate and drying time was statistically significant, even when the airflow rate was changed to 1.45 m/s. The drying curves drawn for the jerky samples dried in the thin layer convection drying unit with 1 m/s airflow rate is shown in Figure 4.7. At the beginning of drying, the moisture removal rate was increased to its highest and a constant rate was maintained for two hours for samples dried at 40% relative humidity and 1 m/s airflow. As in lower relative humidity samples, there was no constant rate period observed. After the first 30 min of drying, the sub surface moisture was removed during a falling rate period.

At 1.45 m/s airflow rate, both samples dried with 40 and 15% relative humidity showed similar patterns of drying (Figure 4.8). At higher airflow rate, the moisture removal was accelerated due to the faster air movement. So, the combined effect of relative humidity and airflow rate left the drying behavior of the beef jerky unchanged. However, the moisture removal rate was higher at 15% relative humidity than at 40%.

Drying curves of beef jerky at different airflow rates and at 40% relative humidity are shown in Figure 4.9. At lower airflow rate, jerky samples dried at a constant rate but for a long period. This improved the drying rate of the samples at the beginning of the drying stage. A falling rate period occurred following by the constant rate periods after 2 and 1.5 h at 1 and 1.45 m/s airflow rate, respectively. The faster moisture removal at the first stage of drying at higher airflow rate resulted in reduced drying time.

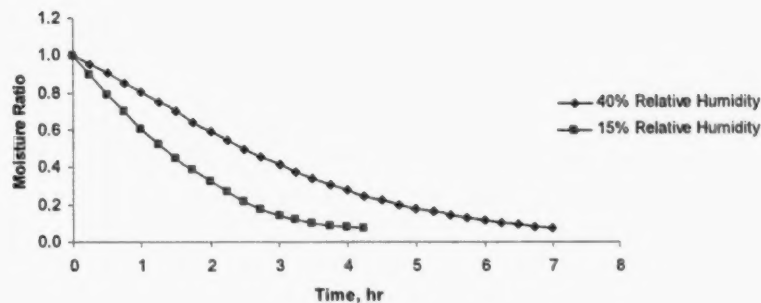


Figure 4.7 Effect of relative humidity on thin layer drying of beef jerky at the air flow rate of 1.00 m/s.

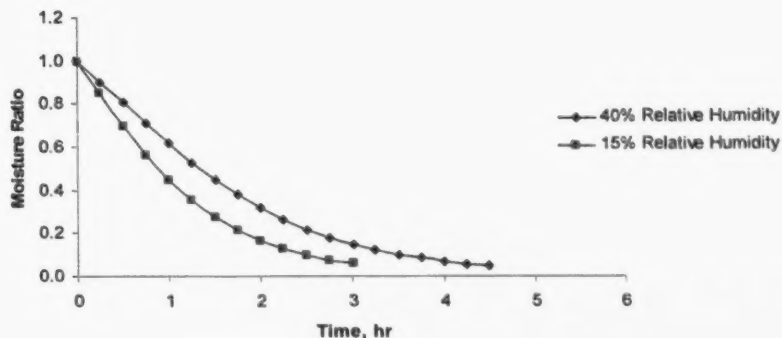


Figure 4.8 Effect of relative humidity on thin layer drying of beef jerky at the air flow rate of 1.45 m/s.

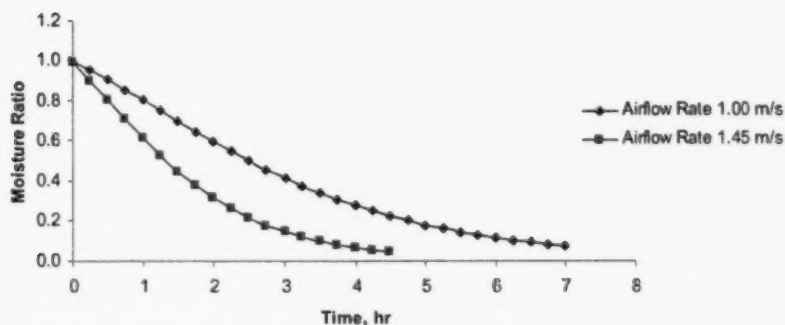


Figure 4.9 Effect of air flow rate on thin layer drying of beef jerky at 40% relative humidity.

4.3.2.2 Physicochemical properties

The shrinkage beef jerky dried in a thin layer drier was significantly increased, when the relative humidity was reduced from 40% to 15%. (Table 4.3). Reducing the relative humidity lowered the water activity from 0.78 to 0.66 in samples dried at 1 m/s, whereas it was reduced from 0.74 to 0.63 in samples dried at 1.45 m/s. There was a significant difference found in shrinkage loss, weight loss and water activity values for samples dried at 40% relative humidity with different airflow rates. The lowest shrinkage and weight loss were measured for the samples dried at higher relative humidity (40%) and lower airflow rate (1 m/s). Water activity values of beef jerky samples dried at different relative humidity levels and airflow rates were within the range of 0.80 to 0.63. These values were within the range of USDA (2003) standards for safety. Water activity values of the samples are significantly reduced, when the airflow rate was increased from 1 m/s to 1.45 m/s. Water activity was also significantly influenced by relative humidity of the heating medium and the air flow rate.

Table 4.3 Physical-chemical properties of beef jerky samples dried under forced-air thin layer dryer

RH. (%)	Airflow Rate (m/s)	Shrinkage (%)	Weight Loss (%)	Water Activity
40	1.00	41.17 ± 1.14 d	62.57 ± 0.09 c	0.80 ± 0.003 a
	1.45	49.31 ± 1.48 c	62.74 ± 0.06 b	0.74 ± 0.007 b
	1.00	58.51 ± 1.34 b	62.90 ± 0.07 b	0.65 ± 0.004 c
15	1.45	60.43 ± 0.22 a	63.72 ± 0.06 a	0.63 ± 0.006 d

Color: Duncan mean differences showed that the color values were significantly changed when a different relative humidity and airflow rate was used to dry the jerky samples. Lightness value was increased when the relative humidity was reduced to 15% from 40% and when the air velocity was increased. The redness value was reduced at the lower relative humidity and higher airflow rate.

Puncture test: The puncture force values were between 16.77 to 42.84 N. The puncture force reported for formed beef jerky by Texture Technologies Corporation was 18.22 N (Texture Technology Corp., 2005). There was a significant effect of relative humidity and airflow rate on puncture force during thin layer drying. The puncture force of the jerky was increased from 16.77 N to 33.93 N when the relative humidity was changed from 40% to 15% at 1 m/s air velocity. This indicates that the sample became harder when the relative humidity was reduced to 15%. Samples also became harder when the airflow rate was increased.

Tensile properties: Tensile properties namely tensile force, elongation and energy required were significantly influenced by relative humidity and airflow rate of the heating medium. The measured tensile force values ranged from 101.7 N to 184.6 N. Tensile forces of the samples dried in the thin layer dryer were lower when compared with the previously reported tensile properties of jerkies. This may be because the sample became harder while drying due to higher airflow rate and lower relative humidity and therefore these samples have possessed higher tensile properties than the others.

4.3.3 Smoke Housing Processing

The moisture removal at the first stage of the drying was high and at this period free water from the surface was evaporated due to conduction. There was no constant rate period of drying observed for jerky processed in the smoke-house. Jerky had a first falling rate period in which most of surface moisture has already evaporated and unbound water molecules try to migrate to the air. This period lasted until the surface moisture becomes zero and at the end of the first falling rate. A second falling

rate period was detected and at this period subsurface moisture removal takes place until it reached the equilibrium moisture content. The average drying rate of this process was calculated as 0.30 db mc/h and average time it has taken was 6.88 h.

Physicochemical properties: Water activity of the sample was observed as 0.71, which is under the food safety recommendations of USDA (2003). Jerky showed a 52.6% shrinkage loss and 57.9% weight loss. These values were significantly lower than that reported in the literature.

4.4 Conclusion

Comparison of drying methods revealed that combined microwave-convection is the best drying method among thin layer drying, smoke-house processing and microwave. It is proven to reduce the shrinkage and weight loss; textural qualities were found to be better. Significant percentage of reduction in time and improved drying rate confirms the possible improvements in processing of jerky using mw hybrid technology. Moreover, the microwave-convection method produced softer jerky compared to other two methods of drying. Thin-layer drying had the capability to produce samples with similar textural characteristics with smoke-house dried ones, which is the representative of commercially available jerky.

Acknowledgements

Meat for this study was provided by the Canadian Cattlemen's Association and the Value Added Beef Program.

ACTIVITY 5 – FEED OPPORTUNITIES FOR THE BIOFUEL INDUSTRIES (FOBI) MEAT QUALITY ASSESSMENT FROM BEEF CATTLE FED BYPRODUCTS OF THE ETHANOL INDUSTRY

5.1 A comparison of wheat and corn-based distiller's grains plus solubles and their combination on the quality of beef *Longissimus*

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Status: Abstract and poster was presented at the Reciprocal Meat Conference 2010 held in Lubbock, Texas, USA. A manuscript is also in preparation for submission to the Canadian Journal of Animal Science.

Abstract:

Western Canada is experiencing a significant increase in wheat-based ethanol production. This has resulted in the generation of large volumes of dried distiller's grains plus solubles (DDGS) that can be used as a feedstock for livestock. Depending on market conditions, wheat and/or corn DDGS may be available. Considerable research has been conducted on the impact of feeding corn DDGS on beef quality, however little is known about the effects of feeding wheat DDGS on beef quality. The objective of this study was to compare meat quality traits of crossbred beef steers fed one of 4 finishing diets. The control diet was a standard barley-based finishing diet comprised of 87.8% barley, 6.5% barley silage and 5.7% supplement (DM basis). The 3 other diets included substitution of 40% wheat (WDDGS), corn (CDDGS), or a 50:50 blend of corn and wheat (BDDGS) based DDGS for barley grain (DM basis). Six-bone rib sections (7th to 12th ribs) from 80 steers (20 per diet) were aged for 14 d and *Longissimus dorsi* steaks were obtained for proximate analysis, drip and cook loss, Warner-Bratzler shear force determination, objective and subjective colour analysis, and oxidative stability analysis (thiobarbituric acid reactive substances- TBARS). Steaks used for colour analysis were wrapped in an oxygen-permeable film and displayed for 7d under simulated retail conditions (1200 lx, 4°C). Ground meat for TBARS was cooked in a water bath to 71°C and stored in the dark for 7d (4°C). Repeated measures analysis using Proc Mixed was used to determine

differences between diets for colour and TBARS, while ANOVAs were used for analysis of the remaining parameters.

No differences ($P < 0.05$) were observed in meat composition, pH, drip loss or shear values due to dietary regime but a trend ($P = 0.07$) was observed for cook loss, with the steaks from animals fed the control diet having the lowest and those from the WDDGS diet having the highest percentage cook loss. Diet had an effect on colour attributes. Steaks from the WDDGS group were lighter (higher L^* value) than steaks from other treatments, while control and CDDGS steaks had similar, intermediate L^* values that varied ($P < 0.001$) from the other diets. Retail display panel results showed WDDGS steaks to have a lighter ($P < 0.001$) lean colour score than the other diets. It was also observed that steaks from animals fed any DDGS diet lost redness faster over time (lower a^* values) and had a less desirable retail appearance than control steaks ($P < 0.001$); however, BDDGS and CDDGS steaks lost redness at a more rapid rate than WDGS. Cooked ground beef from animals fed any of the DDGS diets had lower ($P = 0.005$) TBARS values than ground beef from animals fed the control diet, with WDDGS and CDDGS ground beef having the lowest values. Overall, feeding 40% WDDGS, 40% CDDGS or their blend did not significantly affect meat quality; however, WDDGS did offer better protection against oxidation than the control, along with enhanced colour stability over CDDGS. In contrast, feeding BDDGS resulted in the poorest colour stability and intermediate protection against oxidation.

5.2 Effect of feeding distillers' dried grains (DDGs) with soluble from corn and wheat on fatty acid composition of flat iron muscle (*Infraspinatus*)

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Status: An article is being prepared for publication

Abstract:

Cattle were fed with five different experimental diets, a control diet, 20% corn DDGs, 20% wheat DDGs, 40% corn DDGs and 40% wheat DDGs. At the end of feeding trials flat iron (*infraspinatus*) muscles (Figure 5.2.1) from both left and right sides of the carcass of 60 animals were excised. Samples were aged for 2 or 20 days and frozen at -80°C for the analyses of fatty acid composition.

Total saturated fatty acid content of flat iron was significantly affected by the aging of muscles but less prominently by feeding (Table 5.2.1 and Table 5.2.2). Saturated fatty acid contents of C14:0, C16:0 and C18:0 were significantly increased compared to the control after feeding DDGs at 20% wheat, 40% wheat, and 40% corn. C17:0 ($P = 0.06$) and C24:0 ($P = 0.07$) saturated fatty acids showed an increasing trend with feeding the same diets as above. The effect of feeding corn DDGs at 20% on saturated fatty acids was similar to feeding the control diet. Total monounsaturated fatty (TMUFA) acids and total polyunsaturated fatty acids (TPUFA) levels of flat iron muscles were not affected by feeding diets containing corn and wheat DDGs. However, C20:1n15 ($P = 0.04$) was detected in flat iron only after feeding 40% wheat DDGs, though levels were very low. PUFA C22:3n6 was slightly increased ($P = 0.009$) only after feeding 40% wheat DDGs but lost during aging. Feeding DDGs containing diets very slightly increased PUFA, C20:3n6 ($P = 0.04$) in fresh meat but the levels decreased with aging of meat. Diets did not have influence on the levels of PUFA C20:4n6 but aging of meat significantly decreased the levels already available.

Feeding cattle with up to a maximum of 40% DDGs of corn and wheat origin slightly increased the PUFA levels in fresh meat. However, during aging PUFA must have chemically modified and become undetectable.

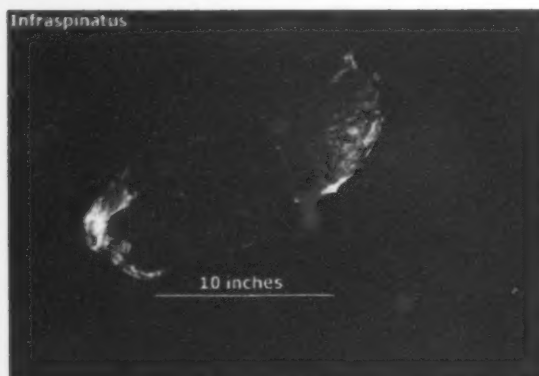


Figure 5.2.1 Flat iron muscle

Table 5.2.1 Effect of DDGs diet on fatty acid composition of flat iron muscles

	Control		20% Corn		20% Wheat		40% Corn		40% Wheat		SEM	P
	Fresh 2d (L)	Frozen 20d (R)	Fresh 2d (L)	Frozen 20d (R)	Fresh 2d (L)	Frozen 20d (R)	Fresh 2d (L)	Frozen 20d (R)	Fresh 2d (L)	Frozen 20d (R)		
Total SFA	43.78	44.33	42.55	43.89	44.06	44.08	42.76	43.36	43.41	44.60	0.71	<0.0001
14:0	3.24	3.39	2.94	3.11	3.04	3.02	3.06	3.11	2.83	2.91	0.12	0.0015
15:0	0.47	0.48	0.47	0.51	0.47	0.47	0.46	0.47	0.49	0.48	0.03	0.3222
16:0	25.17	25.57	23.76	24.31	24.52	24.32	23.56	23.61	24.35	25.10	0.49	0.0059
17:0	1.33	1.35	1.17	1.22	1.16	1.17	1.02	1.03	1.22	1.23	0.06	0.0610
18:0	13.24	13.26	13.19	14.54	14.59	14.80	14.39	14.97	14.20	14.68	0.41	<0.0001
24:0	0.32	0.26	0.30	0.20	0.23	0.24	0.24	0.17	0.30	0.12	0.06	0.0733
Total MUFA	48.58	48.06	46.11	46.12	46.31	45.92	46.27	45.41	45.19	45.61	0.67	0.1702
c-14:1n5	0.72	0.73	0.68	0.68	0.64	0.63	0.57	0.57	0.59	0.58	0.04	0.7295
c-15:1	0.87	0.69	0.55	0.55	0.67	0.61	0.50	0.51	0.63	0.37	0.11	0.0865
n-16:1	0.17	0.23	0.13	0.22	0.13	0.21	0.19	0.22	0.20	0.17	0.04	0.0515
c-16:1n7	3.52	3.57	3.02	2.95	3.06	2.99	3.08	2.95	2.82	2.83	0.14	0.1342
c-17:1 n6	1.12	1.15	0.87	0.85	0.84	0.85	0.75	0.72	0.85	0.83	0.87	0.6815
c-18:1n9	39.27	38.20	39.01	39.04	39.13	38.82	39.43	38.85	38.30	39.35	0.63	0.3870
c-18:1n12	2.85	3.41	1.82	1.81	1.79	1.72	1.63	1.57	1.67	1.63	0.08	0.0997
c-20:1n9	0.06	0.10	0.02	0.03	0.04	0.10	0.04	0.03	0.10	0.07	0.03	0.4001
c-20:1n15	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.03	0.03	0.01	0.0418
Total PUFA	5.74	5.45	8.69	7.46	7.50	7.43	8.44	8.38	16.62	7.93	2.45	0.1846
n-18:2	0.34	0.39	0.36	0.36	0.30	0.30	0.27	0.25	0.32	0.31	0.04	0.8775
c-18:2n6	3.97	3.72	6.54	5.76	5.64	5.56	6.74	6.79	6.96	6.16	0.41	0.0245
c-18:3n3	0.38	0.41	0.44	0.44	0.50	0.49	0.41	0.43	0.59	0.59	0.03	0.5485
c-20:2n6	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.03	0.01	0.7520
c-20:3n6	0.14	0.11	0.25	0.20	0.24	0.24	0.20	0.16	0.27	0.12	0.05	0.0432
c-20:4n6	0.91	0.78	1.03	0.75	0.85	0.85	0.77	0.75	0.85	0.54	0.11	0.0158
c-22:3n6	0.04	0.00	0.05	0.00	0.01	0.02	0.04	0	0.06	0.00	0.02	0.0090
Total Unidentified FA	1.92	2.15	2.65	2.52	2.13	2.50	2.56	2.85	2.30	1.85	0.17	0.5706

Table 5.2.2 Effect of aging on fatty acid composition of flat iron muscles

	Aging			
	Fresh 2d (L)	Frozen 20d (R)	SEM	P
Total SFA	43.31	44.05	0.17	<0.0001
14:0 (F1)	3.02	3.11	0.05	0.0015
15:0 (F3)	0.47	0.48	0.01	0.3222
16:0 (F5)	24.27	24.57	0.22	0.0059
17:0 (F8)	1.18	1.20	0.03	0.0610
18:0 (F10)	14.07	14.45	0.18	<0.0001
24:0 (F22)	0.26	0.21	0.03	0.0733
Total MUFA	46.49	46.22	0.30	0.1702
c-14:1n5 (F2)	0.64	0.63	0.02	0.7295
c-15:1 (F4)	0.65	0.55	0.05	0.0865
t-16:1 (F6)	0.16	0.21	0.02	0.0515
c- 16:1n7 (F7)	3.10	3.06	0.06	0.1342
c- 17:1 n6(F9)	0.89	0.88	0.02	0.6815
c- 18:1n9 (F11)	39.02	38.85	0.28	0.3870
c- 18:1n12 (F12)	1.95	2.03	0.04	0.0997
c- 20:1n9 (F17)	0.05	0.06	0.01	0.4001
c- 20:1n15 (F16)	0.02	0.00	0.00	0.0418
Total PUFA	7.91	7.33	1.09	0.1846
t- 18:2 (F13)	0.32	0.32	0.02	0.8775
c- 18:2n6 (F14)	5.97	5.60	0.18	0.0245
c- 18:3n3 (F15)	0.47	0.47	0.01	0.5485
c- 20:2n6 (F18)	0.01	0.01	0.01	0.7520
c- 20:3n6 (F19)	0.22	0.17	0.02	0.0432
c- 20:4n6 (F20)	0.88	0.73	0.05	0.0158
c- 22:3n6 (F21)	0.04	0.00	0.01	0.0090
Total Unidentified FA	2.31	2.37	0.07	0.11

ACTIVITY 6: PRODUCT DEVELOPMENT, EXTENSION AND TECHNOLOGY TRANSFER

6.1 Product and Process Development

The Value-Added Beef program built on the strengths and expertise of the former ADF-funded Value-Added Meats Strategic Research Area (Saskatchewan Food Product Innovation Program, SFPIP) and provided some financial support for their activities as the meat processing specialist was partially funded from this initiative. SFPIP staff carried out much of the ongoing beef product and process development work. Work often extended beyond the bench-top or pilot scale, with staff providing assistance with scale-up of these new products at the plant level or in cooperation with the Saskatchewan Food Industry Development Centre. Several new and novel beef products have now been commercialized.

In general, product and process development was performed as a response to a market need and demands from the beef industry. There has been a steady demand for the development of value-added products and processing techniques to accommodate the beef sector. Most product development initiatives involved utilizing low value cuts of meat and the trimmings with an aim to increase the overall value of the carcass. Low value cuts have been used to produce whole muscle products, while trimmings are being utilized in the production of restructured and comminuted meat products. A large focus of development had been in the snack food market; examples include jerky, pepperoni and beer sausage. Other deli products and specialty items such as salamis, smoked sausage and garlic sausage had also seen a continuing demand. Determining the shelf life of fresh retail cuts during transportation and ready-to-eat products for the hotel restaurant institution (HRI) trade was also explored.

Over the past eight years several product development projects were undertaken with producers, processors, associations and cooperatives. Several clients came in with only a vague idea of what they wanted. We have been able to help develop their concept and to prepare product prototypes. All these projects were aimed for the domestic market, and where applicable, some for export markets. Products have included acidified beef sticks, some prototypes of burgers/patties, marinated cuts (inside and outside rounds) and various sausages. While some were prepared with beef alone, many were beef/pork combinations. Some of the projects were carried out by request of the Saskatchewan Food Industry Development Centre. Some of the products were showcased at the 2006 windup reception for the Saskatchewan Beef Development Fund.

Many beef processors were looking for ways to produce acceptable products rather than grind the entire animal into "hamburger." Many processors also encountered problems with products which required ingredient or process changes to meet the ever-changing demands of consumers. We had also developed prototypes of burgers and marinated cuts and sausages for a Saskatchewan beef producer who is developing a market for Natural Angus beef products free from antibiotics.

6.2 Extension / Technology Transfer /Education

Information was disseminated via publications, seminars, meetings, workshops, newsletters, etc (Appendix 1 and 2). Several information sheets were developed. The various workshops provided are also listed in Appendix 2.

We also assisted in the expansion and diversification of the Saskatchewan meat processing industry through education and training of Saskatchewan meat processors. Over the past years, several short courses for individuals with interest in the meat processing sector were provided or coordinated by VAB and SFPIP. For example, in 2009, we delivered two workshops to a beef processor to provide training for their staff on burger processing.

Personnel from the program attended a number of trade shows, conferences and meetings etc. during the past eight years. These included Canadian Cattleman's Association Annual Conventions, Saskatchewan Beef Symposium, Canadian Meat Science Association / Canadian Meat Council's Annual Technology Symposiums, Annual Conventions of Saskatchewan Meat Processors' Association, Western Beef Development Centre Termuende Annual Field Days, Grand Opening of the Saskatchewan Food Industry Development Centre, Reciprocal Meat Conference and International Congress of Meat Science and Technology. Important new contacts were made during these meetings.

Having research and product development infrastructure in place for beef may also have helped to attract new business to the province. In 1999-2000, we participated in several information sessions with Saskatchewan Economic Development to highlight Saskatchewan's meat research and development capability for a company considering building a new beef patty processing facility in Saskatoon. While our role was very minor, the province was successful in attracting this company to Saskatoon, with the creation of 100 plus jobs and a need for many tones of meat (beef in particular). In recent years, we then responded to several requests for technical assistance, staffing and training.

We have strived to remain up-to-date with the current needs of the beef industry. For example, our staff have been on the board of directors of the Saskatchewan Meat Processors Association and also on the advisory board for SIAST's Retail Meat Cutting Program. Further, Dr. P. Shand has participated in several events for the Canadian Cattlemen's Association and the Beef Information Centre. She contributed to several of their priority setting events. Since 2004, she participated in the Commercial Beef Technical Advisory Group, which was a special task force set up by the Beef Information Centre. The group provided input on research and technology needs for Canadian processors following the BSE crisis. In July 2010, she was invited to present an overview of palatability enhancement through further processing to the CCA Canadian Beef Quality Symposium.

Acknowledgements

Thanks are expressed to Dr. Zeb Pietrasik, Beef Scientist who was with the VAB program from 2001 to 2007 and to the graduate students (A. Perera, V. Thiagarajan and L. Stoll) who were partially supported by the program. Also, I acknowledge the contributions of P. Rogers and D. Pobereznek who carried out much of the beef product development. Technical assistance was provided by H. Silcox, N. Wiebe, D. Moore, E. Pietrasik, R. Sefton and T. Sanjeewa. We are also grateful for the technical assistance and research support provided by the Saskatchewan Food Industry Development Centre and Agriculture and Agri-Food Canada, Lacombe Research Centre.

APPENDIX 1: Publications, Abstracts and Conference Proceedings

Peer-Reviewed Journal Articles

Published

Pietrasik, Z. and Shand, P.J. 2010. Effects of moisture enhancement, enzyme treatment and blade tenderization on the processing characteristics and tenderness of beef *semimembranosus* steaks. *Meat Science*. Accepted.

Pietrasik, Z. and Shand, P.J. 2010. Bovine *biceps femoris* is resistant to tenderization by lower-salt moisture enhancement or enzyme addition. *Can. J. Animal Science*. Accepted.

Pietrasik, Z., Aalhus, J.L., Gibson, L.L. and Shand, P.J. 2010. Influence of blade tenderization, moisture enhancement and pancreatin enzyme treatment on the processing characteristics and tenderness of beef *semitendinosus* muscle. *Meat Science*. 84: 512-517.

Boles, J. A. and Shand, P.J. 2008. Effect of muscle location, fiber direction, and slice thickness on the processing characteristics and tenderness of beef stir-fry strips from the round and chuck *Meat Science*. 78: 369-374.

Pietrasik, Z., Dhanda, J.S., Shand, P.J. and Pegg, R.B. 2006. Influence of injection, packaging, and storage conditions on quality of beef and bison steaks. *Journal of Food Science* 71:5110-118.

Janz, J.A.M., Pietrasik, Z., Aalhus, J.L. and Shand, P.J. 2005. The effects of enzyme and phosphate injections on the quality of beef *semitendinosus*. *Canadian Journal of Animal Science*, 85: 327-334.

Pietrasik, Z. and Shand, P.J. 2005. Effects of mechanical treatments and moisture enhancement on the processing characteristics and tenderness of beef *semimembranosus* roasts. *Meat Science*, 71: 498-505.

Manuscripts submitted or in preparation

Perera, A., Pietrasik, Z. and Shand, P.J. 2010. An investigation on heat induced physicochemical, mechanical and structural changes of bovine epimysium. In preparation for submission to *Food Chemistry*.

Pietrasik, Z. and **Shand, P.J.** 2010. Effect of postmortem tenderization strategies (pre-tumbling, blade tenderization, moisture enhancement) on processing yield parameters and eating quality of selected hip and loin muscles from youthful and mature beef carcasses. In preparation for submission to Journal of Animal Science.

Stoll, L.C., **Shand, P.J.**, AAlhus, J., Dugan, M. and McKinnon, J.J. 2011. A comparison of wheat, corn, and blend-based distiller's grains plus solubles on the quality of beef *Longissimus*. In preparation for submission to Canadian Journal of Animal Science.

Abstracts and Conference Proceedings

Stoll, L.C., **Shand, P.J.** and McKinnon, J.J. 2010. A comparison of wheat, corn, and blend-based distiller's grains plus solubles on the quality of beef *Longissimus*. 63rd Reciprocal Meat Conference of the American Meat Science Association, Lubbock, Texas, June 20-23 [poster LCS and PJS] Abstract-Meat Science TBD

Lepetit, J. and **Shand P.J.** 2009. Effect of heating on the transverse adhesion of collagen fibers. Proceedings of the 55th International Congress of Meat Science and Technology, Copenhagen, Denmark, August 16-21. PE7.15 pp. 1138-1141. [short paper and poster]

Perera*, A., Olkowski, A.A. and **Shand P.J.** 2009. Efficacy of sodium and ammonium hydroxide as connective tissue tenderizers. Proceedings of the 55th International Congress of Meat Science and Technology, Copenhagen, Denmark, August 16-21. PE7.16 pp. 1141-1146. [short paper and poster]*Ph.D. student

Perera*, A., Pietrasik, Z. and **Shand, P.J.** 2009. Effect of strong and weak acids on the structural integrity of bovine epimysium. Canadian Meat Science Association / Canadian Meat Council Joint Technical Symposium, May 7, Montreal, Quebec. Abstract-proceedings. [poster PJS] *graduate student

Perera*, A., Pietrasik, Z. and **Shand, P.J.** 2008. Effect of strong and weak acids on the structural integrity of bovine epimysium. Institute of Food Technologists Annual Meeting, New Orleans, LA. Abstract, page ?. [poster A.P.] *graduate student

Pietrasik, Z. and **Shand, P.J.** 2008. Effect of aspartic protease from *Aspergillus Oryzae* on the tenderness of beef. Canadian Meat Council Annual Conference, May 6-9, Niagara Falls, ON abstract [poster ZP]

Pietrasik, Z., Gibson, L.L., Aalhus, J.L. and **Shand, P.J.** 2007. Effects of moisture enhancement, enzyme treatment and blade tenderization on the processing characteristics and tenderness of beef *semimembranosus* steaks. 60th Annual Reciprocal Meat Conference of the American Meat Science Association, June 17-20, Brookings, SD. Abstract # 21 [poster PJS].

Perera*, K.A.A., Pietrasik, Z. and Shand, P.J. 2007. Effects of heating time and temperature on shear force and physiochemical characteristics of bovine *longissimus* epimysium. 60th Annual Reciprocal Meat Conference of the American Meat Science Association, June 17-20, Brookings, SD. Abstract # 79 [oral KP] *graduate student.

Pietrasik, Z. and Shand, P.J. 2006. Effect of aspartic protease from *Aspergillus Oryzae* on the tenderness of beef. Proc. 52nd Int. Conf. Meat Sci. Technol., August 13-18, Dublin, Ireland. extended abstract p. 475-476. [poster ZP, PJS].

Shand, P.J., Chambers IV, E. and Hunt, M.C. 2005. Flavor and texture profiling of enhanced beef *longissimus*. 51st International Congress of Meat Science and Technology August 7-12, Baltimore, Maryland, abstract, (paper also on CD pp 507 -510).

Thiagarajan, I.V., Meda, V. and Shand, P. 2005. Effect of pH on microwave drying and textural characteristics of beef jerky. 2005 North Central ASABE/CSBE Conference. Brookings, South Dakota, Sept 29-Oct 1. Paper #SD05-901, 11 pp.

Factsheet and non refereed articles:

Z. Pietrasik and P.J. Shand. 2004. Manipulating brine addition to improve water binding and textural characteristics of cooked, restructured beef rolls. CMSA news. December.

Shand, P.J., Z. Pietrasik and Price, M. 2007. Processing interventional strategies to enhance tenderness of underutilized beef cuts. BCRC Research Review Factsheet. September

Pietrasik, Z. and Shand, P. 2006. Research summary fact sheet on "Effect of postmortem tenderization strategies (enhancement, blade tenderization and other tenderization methods) on properties of selected cow muscles". 1 pp. Circulation: 500

Media reports:

"Responding to changing customer habits with beef." Farm & Food Report, Communications Branch, Saskatchewan Agriculture Food and Rural Revitalization, February 7, 2005. log # 05-06-030

"Beef industry researchers are looking into ways to make beef more tasty and more tender to keep and attract consumers." Canada Now (CBKT-TV), February 9, 2005 Length- 2:03. Reach- 4,000. (TV interview on site)

"Beef industry researchers are looking into ways to make beef more tasty and more tender to keep and attract consumers." 16:30 News (CBK-AM), February 9, 2005 Length- 1:23. Reach- 13,000. (radio interview)

"Interview regarding research underway to discover ways to make cuts of beef more attractive and easier and faster to prepare to accommodate busy consumers, such as pre-cooked products." Noon Edition (CBK-AM)), February 9, 2005 Length- 7:00. Reach- 19,000. (radio interview)

" Saskatchewan looking for ways to make beef more tender so it will taste better and try to increase profits in industry" CBC News, Countrywide – AM, Digital Cable, February 16, 2005. Length – 2:00 Reach- 1, 000. (TV clip)

"Question: what is it in pemmican that keeps it preserved?" P. Shand was an invited participant on CBC Radio's Quirks and Quarks Question show. Taped June 1, 2005 in front of a live audience; aired June 4. Reach 500,000

APPENDIX 2: Lectures, Presentations and Workshops

Invited Lectures and Conference Presentations

Shand, P.J. 2010. Processing interventions to enhance beef quality. Invited speaker at the Canadian Beef Quality Symposium, July 8, Calgary, AB

Shand, P.J. 2008. Adding value to underutilized beef cuts. Invited speaker at the Saskatchewan Beef and Forage Symposium, February 6-7, Saskatoon.

Shand, P.J. 2007. Enhancing eating quality of beef from mature cows. Seminar to the Saskatchewan Sustainable Beef Systems Research Group, April 19, 2007. Saskatoon.

Shand, P.J., 2006. Enhancing the eating quality of beef from mature cows. 2006 Saskatchewan Beef Symposium, February 8, 2006. Saskatoon, Sask.

Shand, P.J., 2005 Commercial beef value-added research. 2005 Commercial Beef Symposium on Enhancing the Utilization of Commercial Beef. Beef Information Centre. presented on October 25 in Mississauga, Ontario and November 1, in Calgary, Alberta. PowerPoint presentation available at www.canadianbeef.info/2005cbsymposium.html.

Short-Courses and Workshops

Burger U- a workshop on the science and technology of burger manufacture. May, 2009 (12 participants) and June, 2009 (14 participants) (7 hours lectures and laboratories)

Processed Meats Seminar- "Producing Winning Products", April 4, 2008

Sausage and Processed Meats Short-Course, May 6-8, 2005. Saskatoon, SK (18 participants) (1.5 hours of lectures)

